

**Osteonecrosis and bone health in children, teenagers and
young people with leukaemia**

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Intellectual property and publications statements

The candidate confirms that the work submitted is her own, except where work which has formed part of jointly-authored publications has been included. The contribution of the candidate and the other authors to this work has been explicitly indicated below. The candidate confirms that appropriate credit has been given within the thesis where reference has been made to the work of others.

The following jointly authored publications have been written as a result of the work in this thesis:

Amin NL, James RM, Phillips R. Should we be using bisphosphonates for osteonecrosis complicating childhood acute lymphoblastic leukaemia? *Archives of Disease in Childhood* 2016; **101**:287-290

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Contributions: N Amin designed the data collection tools, developed the methodology, acquired, analysed and interpreted the data, and wrote and revised the manuscript. B James and S Kinsey helped with design of the data collection tools, methodology, and edited the manuscript. R Feltbower provided advice in data analysis, and revised the manuscript. A Vora helped with editing the manuscript.

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Contributions: N Amin contributed to protocol development and wrote and revised the manuscript. All authors contributed with protocol development, and editing of the manuscript.

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Abstract

Following a review of the literature describing the bone health of children, teenagers and young people with leukaemia, this thesis is comprised of two main parts. The first part describes a retrospective review of patients with acute lymphoblastic leukaemia (ALL) who were recruited into the national study, UKALL 2003. This reports upon the UK prevalence of symptomatic osteonecrosis (ON) in young people with ALL, assessing the chronology of development of symptoms and subsequent diagnosis. This study also evaluated risk factors for the development of ON, and determined the joints most commonly affected. The surgical and medical management of patients is described, with a review of long-term outcomes of patients.

This is the largest single UK study reporting symptomatic ON in childhood ALL, providing long term follow up data of patients. The overall prevalence of symptomatic ON was calculated to be 5.5%. Age at diagnosis of ALL significantly affected risk of development of ON, with the highest risk in those aged between 10 and 20 years at diagnosis of ALL. Affected patients had a high rate of surgical intervention, with hip replacements in 26% of patients. Core decompression was performed in 30% of hips affected by ON but we found no significant difference in femoral head survival between those patients who had core decompression compared with conservative management

The second part of this thesis describes the establishment and interim findings of the British OsteoNEcrosis Study, a prospective longitudinal cohort study of patients aged 10-25 years diagnosed with ALL or lymphoblastic lymphoma. This is the first multi-centre prospective study using MRI imaging for assessment of asymptomatic ON in the UK, and combines physiotherapy assessment with imaging and biochemical results. The results suggest osteonecrotic lesions develop between induction and start of maintenance chemotherapy, with the majority of patients developing multiple asymptomatic osteonecrotic lesions by the start of maintenance chemotherapy.

Contents

Intellectual property and publications statements	I
Acknowledgements	III
Abstract	IV
Contents	V
List of tables	X
List of figures	XII
List of abbreviations	XIV
Chapter 1 Introduction	1
1.1 Normal bone anatomy and physiology.....	3
1.1.1 Bone remodelling.....	5
1.1.2 Endocrine regulation of bone metabolism	6
1.2 Bone fragility	7
1.2.1 Bone mineral density and DXA interpretation.....	8
1.2.2 Alternative methods of measuring bone mineral density	9
1.2.3 Vertebral fracture detection.....	10
1.3 Osteonecrosis and the role of glucocorticoids	11
1.4 Glucocorticoids and bone fragility.....	13
1.5 Background of current UK treatment for acute lymphoblastic leukaemia	15
1.6 Current treatment for acute lymphoblastic leukaemia	16
1.7 Glucocorticoid therapy in acute lymphoblastic leukaemia	21
Chapter 2 Literature review	23
2.1 Osteonecrosis in young people with acute lymphoblastic leukaemia	23
2.1.1 Research questions	23
2.1.2 Search strategies.....	23
2.1.3 Prevalence and incidence of osteonecrosis in children and young adults being treated for acute lymphoblastic leukaemia at different time points in their treatment	39
2.1.4 Current classification systems for osteonecrosis.....	41
2.1.5 The natural history of osteonecrosis in young patients with acute lymphoblastic leukaemia	48
2.1.6 Risk factors for development and progression of osteonecrosis in young people with acute lymphoblastic leukaemia	49
2.1.7 Summary	59

2.2 Bone mineral density changes in children and young people with acute lymphoblastic leukaemia	61
2.2.1 Research questions	61
2.2.2 Search Strategies	61
2.2.3 Bone mineral density at diagnosis and during treatment of acute lymphoblastic leukaemia	68
2.2.4 Bone mineral density of patients treated for acute lymphoblastic leukaemia after completion of therapy	69
2.2.5 Risk factors for low bone mineral density	70
2.2.6 Fracture risk of patients during and after treatment for acute lymphoblastic leukaemia	71
2.2.7 Risk factors associated with increased fracture rate in patients with acute lymphoblastic leukaemia.....	73
2.2.8 The natural history of vertebral fractures in patients with acute lymphoblastic leukaemia	74
2.2.9 Summary	74
2.3 Potential therapeutic strategies	76
2.3.1 Bisphosphonate therapy in the management of young patients with osteonecrosis and acute lymphoblastic leukaemia	76
2.3.2 Efficacy and safety of vitamin D in children and young people with acute lymphoblastic leukaemia: a systematic review	86
Chapter 3 A retrospective national study of osteonecrosis in children and young people with acute lymphoblastic leukaemia.....	107
3.1 Introduction	107
3.2 Methods	107
3.2.1 Patient population	107
3.2.2 Questionnaire development	111
3.2.3 Data Source.....	113
3.2.4 Questionnaire distribution	113
3.2.5 Managing returns and data collection	114
3.2.6 Statistical analysis	114
3.2.7 Ethical permission.....	116
3.3 Results	116
3.3.1 Prevalence of osteonecrosis in UKALL 2003	116
3.3.2 Chronology of symptomatology and diagnosis of osteonecrosis in patients with acute lymphoblastic leukaemia ..	118
3.3.3 Risk factors associated with development of osteonecrosis.....	119

3.3.4 Method of diagnosing osteonecrosis.....	123
3.3.5 Joints affected by osteonecrosis	123
3.3.6 Medical management of patients	124
3.3.7 Surgery in patients with osteonecrosis.....	126
3.3.8 Long-term outcomes of patients affected by osteonecrosis.....	128
3.4 Detailed analysis of surgical and radiographic outcomes in children and young adults in UKALL 2003 affected by osteonecrosis	130
3.4.1 Introduction.....	130
3.4.2 Methods.....	130
3.4.3 Results: Part A.....	132
Surgical management of patients with osteonecrosis	132
3.4.4 Results: Part B.....	139
Efficacy of core decompression of the femoral head.....	139
Chapter 4 The British OsteoNEcrosis Study.....	142
4.1 Study development.....	142
4.1.1 Introduction.....	142
4.1.2 Consultation phase	144
4.1.3 Key points emerging from consultations with professionals	146
4.1.4 Development of study literature	154
4.1.5 Study development following consultations with families.....	154
4.1.6 Study promotion	155
4.1.7 Data management strategy.....	155
4.1.8 Statistical analysis	155
4.1.9 Summary of amendments.....	156
4.2 The British OsteoNEcrosis Study Protocol.....	157
4.2.1 Objectives.....	157
4.2.2 Study design.....	157
4.2.3 Study setting.....	157
4.2.4 Study population.....	158
4.2.5 Recruitment target	158
4.2.6 Study outcomes.....	158
4.2.7 Patient assessment	158
4.2.8 Data analysis	160
4.2.9 Data management and ethical permission	162
4.3 Results	163
4.3.1 Patient demographics	163

4.3.2 MRI and physiotherapy results.....	165
4.3.3 Bone mineral density and vertebral fractures	171
Chapter 5 Discussion	174
5.1 A retrospective review of the UKALL 2003 cohort.....	175
5.1.1 UK prevalence of symptomatic osteonecrosis.....	175
5.1.2 Chronology of development of symptoms and diagnosis of osteonecrosis.....	175
5.1.3 Risk factors for the development of symptomatic osteonecrosis.....	176
5.1.4 Joints affected by symptomatic osteonecrosis and methods of diagnosis.....	179
5.1.5 Methods of diagnosis of osteonecrosis	179
5.1.6 Medical management of patients diagnosed with osteonecrosis.....	179
5.1.7 Surgical management of patients diagnosed with osteonecrosis.....	181
5.1.8 Efficacy of core decompression in prevention of joint collapse	184
5.1.9 Long-term outcomes of patients affected by osteonecrosis.....	185
5.1.10 Strengths and limitations.....	186
5.2 Development of a protocol to establish a prospective longitudinal cohort of young people with acute lymphoblastic leukaemia of lymphoblastic lymphoma	189
5.2.1 Challenges.....	189
5.2.2 Analysis of preliminary results.....	189
5.2.3 Future data management plan.....	191
5.2.4 Strengths and limitations.....	192
Chapter 6 Conclusion.....	194
6.1 Main findings	194
6.2 Clinical implications	195
6.3 Ongoing and future work	195
6.4 Overall conclusion	198
Appendices	199
Appendix 1. Chemotherapeutic agents used during treatment in UKALL2011 in those eligible for BONES:	199
Appendix 2. Data extraction form for systematic review assessing efficacy and safety of vitamin D in children and young people with acute lymphoblastic leukaemia.....	202

IX

Appendix 3. Questionnaire distributed for UKALL2003 ON data collection	209
Appendix 4. Additional form for identification of unreported cases of ON in UKALL2003.	212
Appendix 5. UKALL2003 Trial Toxicity reporting form (page 1)	213
Appendix 6. Stata code for UKALL2003 data analysis.....	214
Appendix 7. Directed acyclic graph codes	216
Appendix 8. Questionnaire responses according to centre	218
Appendix 9. Questionnaire for surgical sub-study of UKALL2003 patients.....	219
Appendix 10. Study protocol for British OsteoNEcrosis Study. Version 5. ..	220
Appendix 11. Patient information sheets and consent forms. British OsteoNEcrosis Study	243
Appendix 12. British OsteoNEcrosis Study ethical approval	250
Appendix 13. Amendments to protocol. Ethical approval.	253
References.....	259

List of tables

Table 1. Retrospective studies reporting prevalence and risk factors for symptomatic osteonecrosis in children and young adults with acute lymphoblastic leukaemia	25
Table 2. Prospective studies reporting prevalence and risk factors for symptomatic osteonecrosis in children and young adults with acute lymphoblastic leukaemia	33
Table 3. Main studies assessing genetic risk factors for development of osteonecrosis in children and young adults with acute lymphoblastic leukaemia	38
Table 4. Modified classification system of Ficat and Arlet	42
Table 5. Classification system of the University of Pennsylvania.....	43
Table 6. Association Research Circulation Osseous classification system.....	44
Table 7. Japanese Investigation Committee classification.....	45
Table 8. St Jude Children's Hospital categorisation for osteonecrosis.....	46
Table 9. Ponte di Legno consensus classification of osteonecrosis	47
Table 10. Studies assessing bone mineral density of patients during treatment for acute lymphoblastic leukaemia	63
Table 11. Studies assessing bone mineral density of patients after completion of acute lymphoblastic leukaemia therapy.....	65
Table 12. Studies assessing fractures in patients with acute lymphoblastic leukaemia	67
Table 13. Studies of bisphosphonate therapy in childhood acute lymphoblastic leukaemia	79
Table 14. Characteristics of studies included for analysis: randomised studies.....	93
Table 15. Characteristics of studies included for analysis: cohort studies	94
Table 16. Risk of bias for randomised controlled trials.	97
Table 17. Risk of bias for non-randomised studies.....	97
Table 18. Changes in vitamin D level after supplementation	100
Table 19. Loke quality assessment for reporting of adverse events	102
Table 20. Grading of recommendations, assessment, development and evaluations evidence profile: vitamin D for children with acute lymphoblastic leukaemia	104
Table 21. Demographic details of patients	117
Table 22. Results of univariable logistic regression analysis of age, sex and ethnicity, and association with osteonecrosis in patients with acute lymphoblastic leukaemia	120

Table 23. Results of multivariable logistic regression analysis of number of blocks of delayed intensification and association with osteonecrosis in patients with acute lymphoblastic leukaemia	121
Table 24. Age by gender interaction for development of osteonecrosis.....	122
Table 25. Surgical procedures reported in patients with osteonecrosis	127
Table 26. Comparison of demographic details for all patients with confirmed osteonecrosis with those for whom secondary questionnaire data was received	133
Table 27. Surgical procedures performed in joints affected by osteonecrosis	134
Table 28. Grading of hip osteonecrosis	139
Table 29. British OsteoNEcrosis Study demographic and biochemical data.....	164
Table 30. Magnetic resonance imaging and physiotherapy results	166
Table 31. Initial bone mineral density and vertebral fracture results	171

List of figures

Figure 1. Structure of a long bone.....	3
Figure 2. Cross-section of a long bone.....	4
Figure 3. Bone composition	4
Figure 4. Proposed pathophysiology for development of osteonecrosis in patients with high dose steroid use	13
Figure 5. UKALL 2011 trial schema for patients over 10 years of age.....	20
Figure 6. Niinimäki osteonecrosis classification system	47
Figure 7. Vitamin D metabolism	87
Figure 8. Study flow diagram of search and selection process.....	92
Figure 9. Outline of UKALL 2003 treatment regimens	110
Figure 10. Treatment and randomisation algorithm in UKALL 2003	111
Figure 11. Directed acyclic graph for causal effect identification in development of osteonecrosis.....	115
Figure 12. Questionnaire response rate for patients with bone toxicity.....	117
Figure 13. Cumulative incidence of osteonecrosis in UKALL 2003.....	119
Figure 14. Age of patients in UKALL 2003 with symptomatic osteonecrosis.	122
Figure 15. Imaging modality used for diagnosis of osteonecrosis.....	123
Figure 16. Number of joints affected in patients with osteonecrosis	124
Figure 17. Joints affected by osteonecrosis	124
Figure 18. Type of bisphosphonates given to patients with osteonecrosis	126
Figure 19. Long term outcomes of patients affected by osteonecrosis who had surgical intervention	129
Figure 20. Directed acyclic graph for causal effect identification of development of grade 5 osteonecrosis.....	132
Figure 21. Joints affected by osteonecrosis in UKALL 2003- surgical sub-study analysis	134
Figure 22. Surgical management of hips affected by osteonecrosis.....	136
Figure 23. Mobility status of patients with osteonecrosis in UKALL 2003	137
Figure 24. Pain status of patients in UKALL 2003 with osteonecrosis	138
Figure 25. Kaplan-Meier failure time estimates for hips comparing core decompression with conservative treatment.....	140
Figure 26. Kaplan-Meier failure time estimates for hips with grade 4 osteonecrosis comparing core decompression with conservative treatment.....	141
Figure 27. Timeline of events for development of British OsteoNEcrosis study ...	143
Figure 28. Schema of British OsteoNEcrosis Study procedures.....	160

Figure 29. Total body less head bone mineral density Z-scores at diagnosis of acute lymphoblastic leukaemia.....	172
Figure 30. Lumbar spine bone mineral apparent density Z-scores at diagnosis of acute lymphoblastic leukaemia.....	172
Figure 31. Joints affected by osteonecrosis: a comparison of patients with confirmed osteonecrosis and those for whom second questionnaire responses were received	182
Figure 32. Joints replacements due to osteonecrosis in UKALL 2003: a comparison of patients with confirmed osteonecrosis and those for whom second questionnaire responses were received	182

List of abbreviations

1,25(OH) ₂ D	1,25 dihydroxycholecalciferol
25OHD	25-hydroxyvitamin D
aBMD	areal bone mineral density
ABQ	algorithm based qualitative
AEIOP	Associazione Italiana di Ematologia e Oncologia Pediatrica
ALL	acute lymphoblastic leukaemia
ARCO	Association Research Circulation Osseous
ASK	Activities Scale for Kids
ASP	asparaginase
BCP ALL	B cell precursor acute lymphoblastic leukaemia
BFM	Berlin-Frankfurt-Munster
BMAD	bone mineral apparent density
BMC	bone mineral content
BMD	bone mineral density
BMI	body mass index
BMP	bone morphogenic protein
BONES	British OsteoNEcrosis Study
CCG	Children's Cancer group
c-HAQ	Childhood Health Assessment Questionnaire
CI	confidence interval
CNS	central nervous system
COG	Children's oncology group
CSF	cerebrospinal fluid
CT	computed tomography
CTX	carboxy-terminal collagen crosslinks
cum inc	cumulative incidence
DFCI	Dana Faber Cancer Institute
DI	delayed intensification
DXA	dual-energy X-ray absorptiometry
EFS	event free survival
EOT	end of treatment
FISH	fluorescence in-situ hybridization
GRADE	Grading of Recommendations, Assessment, Development and Evaluations
GWAS	genome wide association studies
HR	high risk
HRpQCT	high resolution peripheral quantitative computerised tomography
IMD	index of multiple deprivation
IQR	interquartile range
IR	intermediate risk
ISCD	International society for clinical densitometry
JAFAS	Juvenile Arthritis Functional Assessment Scale
LBL	lymphoblastic lymphoma
LEFS	Lower Extremity Functional Scale

LSBMD	lumbar spine bone mineral density
M-CSF	macrophage colony-stimulating factor
MRC	Medical Research Council
MRD	minimal residual disease
MRI	magnetic resonance imaging
MSC	mesenchymal stem cells
NCI	National Cancer Institute
NELL1	neuroepidermal growth factor-like 1
NHL	non-Hodgkin's lymphoma
NOPHO	Nordic Society for Paediatric Haematology and Oncology
ON	osteonecrosis
OPAL	Osteonecrosis in Paediatric patients with Acute lymphoblastic Leukemia and Lymphoblastic Lymphoma
OR	odds ratio
P1NP	N-terminal pro-peptide of type 1 procollagen
PedsQL	Paediatric Quality of Life Inventory Scale
PODCI	Paediatric Outcomes Data Collection Instrument
pQCT	peripheral quantitative computerised tomography
PTC	Primary treatment centre
PTH	parathyroid hormone
QCT	quantitative computerised tomography
QuEST	Quality of life Evaluation in patients receiving Steroids
RANKL	receptor activator of nuclear factor kappa-B ligand
RHR	relative hazard ratio
ROBINS I	Risk of bias in non-randomised studies of interventions
SDS	standard deviation score
SIOP	Société Internationale d'oncologie Pédiatrique
SJCRH	St Jude Children's Research Hospital
SNP	single nucleotide polymorphisms
SR	standard risk
STIR	short tau inversion recovery
STOPP	Steroid Associated Osteoporosis in the Paediatric Population
TBBMD	total body bone mineral density
TBLH	total body less head
THR	total hip replacement
VDR	vitamin D receptor
VF	vertebral fracture
WCC	white cell count

Chapter 1 Introduction

Historically, the research agenda for young people with cancer has been set by researchers and professionals caring for young people with cancer. However, there is increasing awareness of the importance of identifying the priorities of the young people and carers themselves. The top 10 research priorities for teenage and young adult were determined by the Teenage and Young Adult Cancer Priority Setting Partnership, a national consultation identifying unanswered research questions by young people, carers, significant others and professionals [1].

Two of the top 10 questions addressed the issue of short and long term effects of cancer treatment. Within the specific field of short and long term side effects, one of the questions was:

“What cancers and treatments cause avascular necrosis, how does it develop, how common is it, what are the physical and psychological effects and what can be done to improve early diagnosis and treatment?”

Whilst the whole of this question is beyond the scope of this thesis, through this work I hoped to move some way towards gaining a greater understanding of how the bone health of children with acute lymphoblastic leukaemia (ALL) is affected, with an emphasis on bone fragility and osteonecrosis (ON), which is also known as avascular necrosis.

Chapter 1 describes bone health, the first part considering normal bone anatomy, physiology and metabolism. The second part of the chapter reflects upon factors that impact bone fragility and the development of ON, specifically with regard to glucocorticoid exposure. The chapter ends with a description of historic and current treatment of ALL, and the use of glucocorticoids in ALL.

Chapter 2 provides an overview of the literature underpinning this thesis. The first part of this chapter describes ON in young people with ALL, followed by a review of bone mineral density changes in this population. The last part of this chapter focusses on efficacy and safety of 2 potential therapeutic strategies, namely bisphosphonate therapy and vitamin D supplementation.

Chapter 3 presents a retrospective analysis of ON in young people with ALL, analysing the cohort of patients enrolled in the national trial for children and young adults with ALL, UKALL 2003. This analysis

- Reports the UK prevalence of symptomatic ON in young people with ALL
- Describes the chronology of the development of symptoms related to ON and subsequent diagnosis of ON
- Identifies risk factors for the development of ON
- Determines which joints are affected by ON and methods of diagnosis of ON in patients with ALL
- Describes the medical and surgical management of patients diagnosed with ON in UKALL 2003
- Establishes the long-term outcomes of patients affected by ON in UKALL 2003

The second part of this chapter includes a subset analysis, focussing on the surgical management of patients, and includes an analysis of the use of core decompression as a therapeutic intervention. The aims of this were to:

- Characterize the surgical procedures performed in patients affected by symptomatic ON in UKALL 2003, including the identification of sequential procedures in individuals.
- Evaluate the efficacy of femoral head core decompression in prevention of joint collapse in young people with symptomatic ON.

Chapter 4 describes the development of a prospective study assessing the natural history of osteonecrotic lesions in young people with ALL and lymphoblastic lymphoma (LBL). Interim results of the study are presented.

The aims of this study are to:

- Identify the incidence of symptomatic and asymptomatic ON in older children, teenagers and young adults being treated for ALL or LBL in the UK at different time points in their treatment
- Identify the risk factors for progression and the development of symptomatic ON in this population
- Identify specific radiological features which might predict for either progression or regression in those with asymptomatic ON
- Evaluate functional ability and explore the correlation of this with MRI findings
- Evaluate changes in BMD and VF incidence during treatment for ALL or LBL

The Chapter 5 is a discussion of the results presented in the preceding 2 chapters, and Chapter 6 concludes with clinical implications and avenues for future research.

1.1 Normal bone anatomy and physiology

The human skeleton is composed of around 270 bones at birth, which decreases to 206 by adulthood (excluding sesamoid bones), as some bones fuse together [2]. Each bone constantly undergoes a process of modelling and remodelling to adapt to changing biomechanical forces and remove micro-damaged bone. The 4 main categories of bones are long bones (e.g. humeri, femurs, tibiae), short bones (e.g. carpal and tarsal bones, patellae), flat bones (skull, mandible, sternum) and irregular bones (vertebrae, sacrum).

Bones are predominantly composed of cortical and trabecular bone (also known as cancellous bone), with different bones having different ratios of cortical to trabecular bone. Long bones have epiphyses at the ends, followed by metaphyses, with the diaphysis (shaft) in the middle (Figure 1) [3]. Cortical bone forms the hard exterior of bones. It is heavily calcified and has a mainly structural and protective role. Trabecular bone is much less dense than cortical bone, is highly vascular and can contain red bone marrow, where haematopoiesis occurs. In long bones the diaphysis (the shaft of the bone) is composed predominantly of cortical bone. In contrast, the metaphysis, which is the area below the growth plate, and the epiphysis, which is above the growth plate, are composed of trabecular bone surrounded by a relatively thin shell of cortical bone (Figure 2) [4]. The vertebrae are composed of predominantly trabecular bone, with a cortical to trabecular bone ratio of 25:75. This ratio is 50:50 in the femoral head and 95:5 in the radial diaphysis [5].

Figure 1. Structure of a long bone

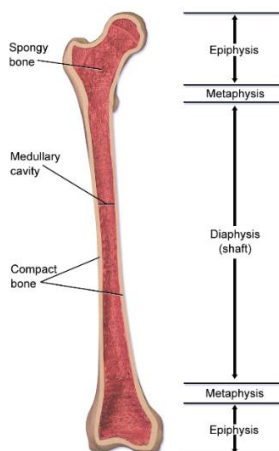
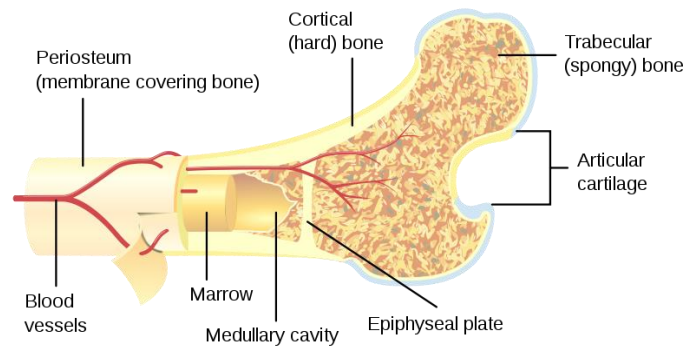


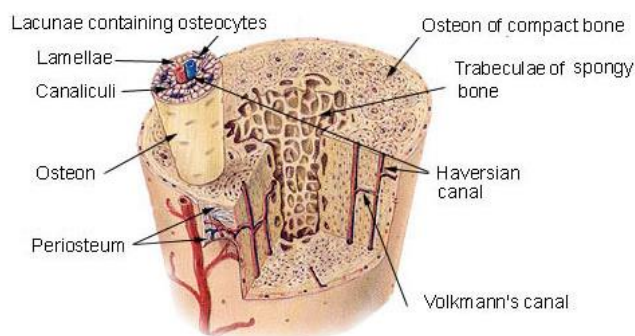
Figure 2. Cross-section of a long bone



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Cortical bone is composed of osteons, whilst trabecular bone is composed of trabeculae. Each osteon is composed of concentric lamellae of compact bone, surrounding a central canal, known as a Haversian canal (Figure 3) [6]. Unlike osteons, trabeculae in general do not have a central canal with a blood vessel. Both cortical and trabecular bone are composed of organic and mineral matrix, with type 1 collagen forming approximately 95% of the organic matrix, and calcium and phosphate ions (in the form of hydroxyapatite) forming the majority of the mineral matrix. The organic matrix provides bone with resistance to tensile forces, whilst the mineral matrix provides bone with strength under compressive loads. Most bones have approximately 60-70% mineral matrix, depending on site and stage of development.

Figure 3. Bone composition



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The vascular supply to bone is critical to ensure it receives adequate oxygenation and nutrient supply, as well as removing metabolic waste products. Around 10% of cardiac output is received directly by the skeleton,

reflecting the requirements of bone cells, marrow, and endothelial cells [7, 8]. The main blood supply of long bones is derived from one or more principle nutrient arteries, which penetrate into the medulla of the bone and connect to the smaller periosteal arteries to enable perfusion [9]. Drainage is into arterio-venous sinuses in the medullary cavity, with exit via multiple small veins that penetrate the cortex. This vascular supply to bone allows the rapid growth and remodelling that differentiates bone from essentially avascular cartilage.

1.1.1 Bone remodelling

Bone remodelling is a continuous process that allows repair of micro-damage and enables skeletal adaptation to mechanical use. It also facilitates maintenance of plasma calcium levels in the physiological range by the release of minerals from the bone matrix. The 3 main cell types involved in the process of remodelling are osteoblasts, osteoclasts and osteocytes. Bone lining cells are also involved in the process of bone remodelling, but their function is not yet fully elucidated. These four cell types form the bone forming unit (BFU), an anatomical structure present during the remodelling cycle.

Osteoblasts are cells located along the bone surface, and their main function is that of bone formation [10]. They are derived from mesenchymal stem cells found in the bone marrow, and produce collagen and other matrix proteins. Commitment of mesenchymal stem cells to osteoblast formation requires activation of the Wnt/ β -catenin pathway [11]. Osteoblasts synthesise new collagenous organic matrix, and regulate matrix mineralisation by the release of membrane bound vesicles that concentrate calcium and phosphate, and destroy mineralisation inhibitors.

Osteoclasts are multinucleated cells that originate from the monocyte/macrophage lineage under the influence of factors including receptor activator of nuclear factor kappa-B ligand (RANKL), secreted by osteoblasts, osteocytes and stromal cells, and macrophage colony-stimulating factor (M-CSF) [12]. The function of the osteoclast is the localised breakdown of bone matrix and mineral. Bone resorption requires osteoclast secretion of hydrogen ions and cathepsin K enzyme. Hydrogen ions acidify the area below the osteoclast to dissolve the mineral component of the bone matrix, whilst cathepsin K digests the organic matrix.

Osteocytes comprise 90-95% of total bone cells, and are terminally differentiated osteoblasts which are incorporated into the bone matrix [13].

Osteocytes connect with one another and the bone surface via multiple cytoplasmic canalicular processes. They are linked through gap junctions and the primary function of the osteocyte-osteoblast/lining cell unit is mechanosensation, transducing stress signals from bend or stretch of bone into biologic activity.

Bone remodelling occurs in both cortical and trabecular bone, and is a sequence of 4 events; activation of osteoclasts, osteoclast mediated bone resorption, reversal and osteoblast mediated bone formation. Osteoclast mediated bone resorption takes approximately 2-4 weeks, and is regulated by the ratio of RANKL to osteoprotegerin, IL-1, IL-6, PTH, 1,25 vitamin D, calcitonin and colony stimulating factor [12]. The subsequent step is reversal, where bone resorption transitions to bone formation. Bone formation takes between 4 and 6 months to complete, and at the completion of bone formation 50-70% of osteoblasts undergo apoptosis, with the remaining cells becoming osteocytes or bone lining cells. The osteoblasts surrounded by and embedded within matrix become osteocytes, with an extensive canalicular network which connects them to the other cells of the bone forming unit [14].

Remodelling can become imbalanced in specific situations, such as in a patient with reduced oestrogen levels or as a consequence of decreased mechanical stimulation [15]. In these conditions there is a net increase in bone breakdown, via relative increases in osteoclast activity, with a concurrent reduction in bone strength and increased fracture risk.

1.1.2 Endocrine regulation of bone metabolism

Many systemic and local hormones influence bone growth and remodelling. As bone is a reservoir of calcium, phosphate, magnesium and trace elements, helping to maintain mineral homeostasis, particularly calcium homeostasis, is one of the functions of bone. The primary hormonal regulators of calcium are parathyroid hormone (PTH) and activated vitamin D (1, 25 dihydroxycholecalciferol, (1,25(OH)₂D)).

In response to hypocalcaemia the parathyroid gland increases the production and secretion of PTH. This acts on the renal tubule to decrease calcium excretion and inhibit phosphate reabsorption, and stimulates 1,25(OH)₂D production [16]. PTH also has direct actions on bone via PTH receptors in osteoblasts, activation of which results in increased calcium and phosphate efflux from the bone fluid compartment [17], and through RANKL

dependent osteoclastic bone resorption [18]. The effects of both PTH and 1,25(OH)₂D result in restoration of normal plasma calcium levels.

When there is sufficient calcium supply, 1,25(OH)₂D can improve calcium balance largely without direct effect on bone cells. However, in calcium deficiency 1,25(OH)₂D enhances bone resorption whilst simultaneously inhibiting bone mineralisation. When the calcium levels normalise 1,25(OH)₂D may provide a drive to re-mineralise the skeleton via action on osteoblastic cells [19].

Calcitonin is a hormone released in response to rising calcium levels, and opposes the effects of PTH. The precise biological role of calcitonin in calcium homeostasis is uncertain as calcitonin deficient patients do not experience alterations in regulation of serum calcium levels.

Oestrogen is an important regulator of bone remodelling, and also has a role in closure of epiphyseal growth plates. Oestrogen receptors are present on both osteoblasts and osteoclasts. Most effects are mediated by the nuclear hormone receptor transcription factors oestrogen receptors α . This is likely to play the dominant role in regulating bone mass in both males and females [20]. Oestrogens act on osteoblasts to increase bone formation and restrict activation of osteoclasts. Loss of oestrogen causes loss of trabecular bone through increased osteoclast numbers.

1.2 Bone fragility

The foundations of adult bone health are developed during childhood and early adult years. Bone strength is determined by peak bone mass, bone size, geometry and microarchitecture, which is primarily established as final height is attained. Heritable factors account for 60-80% of variation in bone strength [21, 22], but in order to achieve one's genetic potential, bone health needs to be optimised during the first 2 decades of life, with peak bone mass established by the third decade of life [23-27]. If this is compromised there may be an associated lifetime risk of osteoporosis and fractures [28, 29]. Whilst in adults osteoporosis is typically defined on the basis of bone mineral density (BMD) assessment [30], the definition is more complicated in the paediatric population.

The International Society for Clinical Densitometry (ISCD) 2013 official paediatric position for diagnosis of osteoporosis is:

“The finding of one or more vertebral compression (crush) fractures is indicative of osteoporosis, in the absence of local disease or high-energy trauma...

In the absence of vertebral compression fractures, the diagnosis of osteoporosis is indicated by the presence of both a clinically significant fracture history and BMD Z-score ≤ -2.0 .” [31].

It is important to note that a vertebral compression fracture (loss of vertebral height at any point of >20%) alone, unless caused by high energy trauma, is sufficient to diagnose osteoporosis, regardless of bone mineral density data due to its implication of significant bone fragility. A clinically significant fracture history is defined as one or more of the following:

- Three or more long bone fractures at any age up to 19 year
- Two or more long bone fractures by age 10 year

Given these guidelines, clinicians require tests that both evaluate bone mineralisation and detect vertebral fractures. Bone densitometry is commonly used to evaluate skeletal mineral status, and aims to identify individuals at risk for skeletal fragility. The most common conventional technique used for non-invasive bone mineral measurements is dual-energy X-ray absorptiometry (DXA).

1.2.1 Bone mineral density and DXA interpretation

Bone densitometry is a surrogate measure of bone strength, with DXA the most commonly used method of assessment due to its widespread availability, low radiation dose and acceptability to the patient. DXA allows measurement of paediatric bone status by measuring the amount of mineral within a given area of bone.

The goal of bone densitometry is to identify individuals at risk for skeletal fragility, determine magnitude of compromised bone mass, and guide and monitor treatment [32]. Although DXA scans are commonly used to assess BMD in patients, the interpretation of BMD results in children requires considerable thought.

The 2013 ISCD official position is that DXA is the preferred method for assessing bone mineral content and areal bone mineral density, with the posterior–anterior lumbar spine and total body less head (TBLH) the preferred sites for measurements [31]. These sites were chosen following results from a study looking at DXA measurements in 450 children with chronic diseases and retrospectively looking at their fracture risk [33]. The

current standard for reporting DXA results is the areal BMD Z-score, which provides an estimate of the standard deviation away from the mean for chronological age and sex [24, 34].

One of the challenges in the interpretation of paediatric DXA measurements is the need to adjust for the influence of bone size. DXA relies on the differential absorption of X-rays to differentiate tissues of different radiographic density and also quantifies the bone mineral content (BMC) at various body sites. The BMD is calculated by dividing the BMC by the bone area. Therefore, DXA-derived BMD is based on the 2-dimensional projected area of a 3-dimensional structure. This will mean that smaller bones will have a lower areal BMD than larger bones, even if the volumetric BMD is the same, and several mathematical models of estimating volumetric BMD have been proposed to negate for the confounding effects of bone size on DXA measurements [35-37].

The ALPHABET study (Amalgamated reference data for size adjusted bone densitometry measurements) is the most recent study to develop UK size adjusted DXA measurements, and has allowed the development of robust reference data for accurate scan interpretation [38]. This study has produced reference curves adjusted for age, sex, ethnicity and body size for lumbar spine bone mineral apparent density (BMAD), lumbar spine areal BMD and TBLH areal bone mineral density. These are applicable for both GE Lunar and Hologic scanners, which are the most common DXA scanners used in the UK, and are validated in patients up to 20 years of age. The prediction equations generated for TBLH BMC also take into account body composition, as several studies have shown a high correlation between muscle mass and bone mass in children, and are consistent with the widely accepted mechanostat theory [39]. The results of the ALPHABET study allow accurate interpretation of UK DXA data, particularly given its UK cohort of 3598 children.

1.2.2 Alternative methods of measuring bone mineral density

Lumbar spine quantitative computerised tomography (QCT), peripheral quantitative computerised tomography (pQCT) and high-resolution pQCT (HRpQCT) are 3-dimensional densitometric techniques that can measure volumetric bone mineral density, distinguish trabecular from cortical components of bone, and determine bone geometry.

As previously discussed, there are significant differences in the microarchitecture of cortical and trabecular bone, with an increased density

of cortical bone compared to the highly vascular trabecular bone. Tibial pQCT and DXA bone Z-scores have been found to be positively correlated, with gains in DXA bone mineral content associated with gains in trabecular volumetric BMD Z-scores [40]. HR pQCT has spatial resolution to measure trabecular geometry and microarchitecture, however is expensive, can only be used to study the peripheral skeleton (tibia, radius) and is currently only used for research purposes due to a lack of standard protocols and normative data.

1.2.3 Vertebral fracture detection

Vertebral fractures (VFs) are a significant marker of bone fragility but may often go unrecognised, and it is estimated that one third of VFs are asymptomatic [41, 42]. Methods of accurate detection of VFs are essential, particularly as it is recognised that fractures are not always associated with reduced bone mineral density as measured by DXA [43]. VFs have historically been assessed by use of lateral spine radiographs, but more recently lateral spine DXA has been shown to be of comparable image quality and diagnostic accuracy [44-46]. Therefore increasing interest has been shown in the use of this imaging modality, due to the significantly reduced radiation exposure to patients, high patient acceptability and availability at the same time as a DXA scan [44].

Among diagnostic protocols to diagnose VFs, the method proposed by Genant is currently one of the most commonly used in clinical practice, with severity of VFs assessed in a semi-quantitative fashion [47]. The fracture is assessed by visual determination of the extent of vertebral height reduction and morphological change, and vertebral fractures are differentiated from other non-fracture deformities. A normal vertebral body is graded 0, a grade 1 deformity is a mild deformity, with moderate and severe deformities classified as grades 2 and 3 respectively. The approximate degree of height reduction determines the assignment of grades to each vertebra. A grade 1 deformity is defined as a 20-25% reduction in anterior, middle and/or posterior height and a reduction in area of 10-20%, with a 25-40% reduction in height in grade 2 fractures, and a 40% reduction in height in grade 3 fractures. Other classification systems, such as the algorithm based qualitative (ABQ) technique have been developed [48], with a simplified ABQ technique described to classify vertebrae as normal, fractured with <25% height loss, fractured with >25% height loss or non-osteoporotic deformity [49]. The threshold of 25% was used as a UK survey of paediatric bone

specialists found that they were most likely to initiate treatment in patients with VFs with a height loss of 25% or more, in the presence of pain [49].

1.3 Osteonecrosis and the role of glucocorticoids

Osteonecrosis is bone death secondary to ischaemia, with all cell types (osteocytes, haematopoietic cells and adipocytes) in the bone and marrow affected.

The nutrient and periosteal blood supply to bone have been described. The nutrient blood supply delivers blood to the medullary cavity and inner half of the cortex, whilst the periosteal blood supply provides vascular support to the external half of the cortex. Both systems provide the most blood to the metaphyseal regions- the growth regions of the bones in children. If either or both systems become impaired the bone will become osteonecrotic. In young children the central portion of the bone, supplied by the medullary blood supply, is occupied by marrow cells (such as polymorphonucleocytes, lymphocytes and monocytes) but in older patients the majority of the cells are lipocytes.

There are a number of known risk factors for ON, but the pathogenesis is incompletely understood as ON is often diagnosed late with no readily accessible bone tissue to sample. However, it is recognised that the earliest pathological characteristics of ON are necrosis of haematopoietic cells and adipocytes, followed by interstitial marrow oedema [50]. In animal models there is osteocyte necrosis after 2-3 hours of oxygen deprivation [51], which is followed by a reactive hyperaemia and revascularisation. This results in bone remodelling that incompletely replaces the areas of bone loss, with bone resorption exceeding formation [52-54]. When this occurs in subchondral trabecular bone there is a loss of structural integrity of the trabeculae, with an associated risk of subchondral fracture.

The loss of vascularity to the subchondral microcirculation may be due to a number of different factors, including:

- Mechanical vascular interruption (post traumatic)
- Intraluminal obliteration (emboli and thrombosis)
- Interosseous extravascular compression
- Direct cytotoxic effects on bone marrow and bone cells.

Mechanical interruption of the blood supply may result from a fracture of the shaft of the bone or from dislocation of a joint. ON due to mechanical

interruption is most common in regions with a blood supply that can easily be completely or partially interrupted by injury, such as in the femoral head. Intraluminal obliteration may result from a number of different mechanisms. Interosseous fat emboli with intravascular coagulation and ON has been described [55], with an overload of subchondral fat emboli, hypercoagulability, stasis and endothelial damage by free fatty acids hypothesised to cause end organ damage. Glucocorticoids causing dyslipidaemia may promote the formation of fat emboli, although fat emboli are also found in healthy bones which do not go on to develop osteonecrosis. The role of hypercoagulability is unclear. Some studies have shown procoagulant abnormalities in patients with ON [56], and thrombophilia-hypofibrinolysis may be a risk factor for development of idiopathic ON [57].

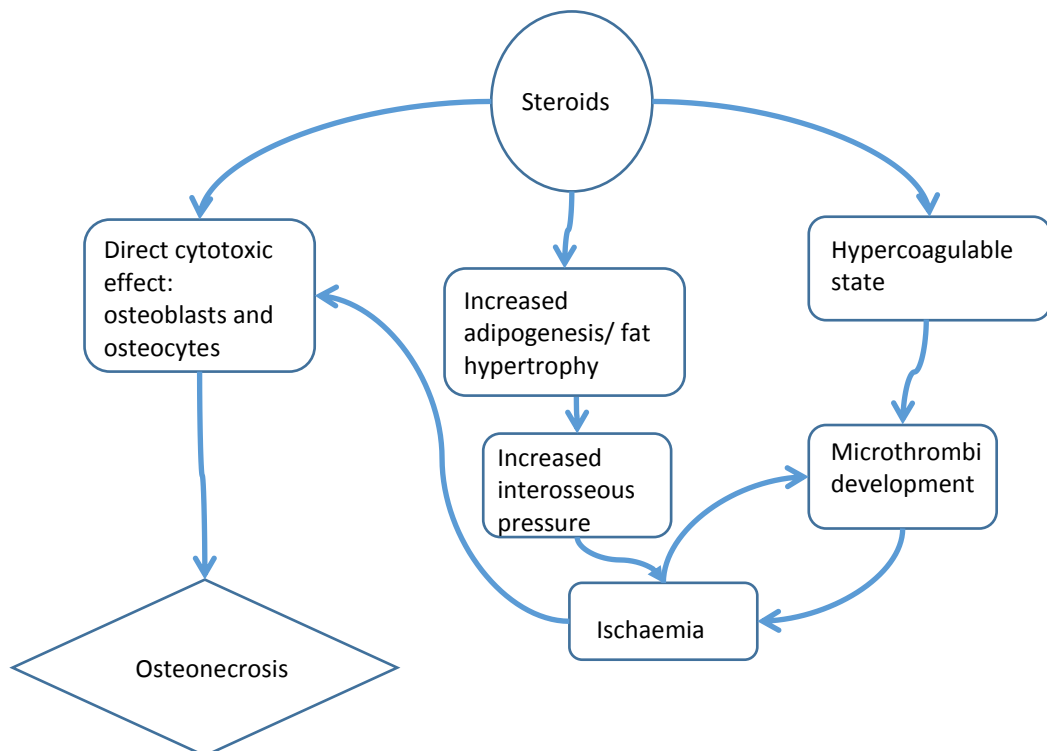
Extraluminal obliteration of blood flow in the intraosseous blood vessels may occur when blood pressure increases within the bone marrow. Lipid deposition and adipocyte hypertrophy are the most likely causes of increases in intraosseous extravascular pressure. Patients with femoral head ON were found to have significantly elevated bone marrow pressures, even before necrosis was detectable [58, 59]. In one animal study it was found that glucocorticoid administration resulted in increased adipocyte size in the bone marrow [60], with a proportionate decrease in intraosseous blood flow, and MRI studies have shown that fat conversion in the marrow occurs in the proximal femur of steroid treated patients, with higher conversion in patients with ischaemic bone lesions [61]. However, elevated intraosseous pressures can be found in other conditions, such as osteoarthritis, which does not lead to development of ON, and it may be that the observed elevations in intraosseous pressure are not causally related to the pathogenesis of ON [62].

Direct cell toxicity can also contribute to the development of ON. Increased osteocyte apoptosis and inhibition of osteoblastogenesis in patients with ON related to glucocorticoid therapy has been suggested in a number of studies [63-65], and reduced replication of osteoblasts may also play a role in glucocorticoid induced ON [66]. Murine models found that mice treated with asparaginase (ASP) treatment alongside dexamethasone had a higher rate of ON than those receiving only dexamethasone after 6 weeks of treatment, with higher rates of epiphyseal arteriopathy observed in mice with dual treatment [67]. A greater exposure to ASP was associated with greater

plasma exposure to dexamethasone, hence this study suggests that ASP could potentiate any osteonecrotic effect of glucocorticoids [67].

It is clear that glucocorticoids can influence the development of ON in a number of different ways, and Figure 4 illustrates a proposed pathophysiology for the development of ON as a result of high steroid use.

Figure 4. Proposed pathophysiology for development of osteonecrosis in patients with high dose steroid use



1.4 Glucocorticoids and bone fragility

The potential role of glucocorticoids in the development of ON has been described, however, glucocorticoids at physiological concentrations are essential for the development of a wide range of tissues. Differentiation of osteoblasts is driven by endogenous glucocorticoids, and glucocorticoid signalling in mature osteoblasts controls skeletal development [68]. However, when exogenous glucocorticoids are administered at pharmacological doses patients can experience bone loss of up to 12% during the first year of therapy [69], resulting in increased bone fragility. Trabecular bone is typically more affected than cortical sites, making spine and rib fractures more common than hip and non-vertebral fractures [70, 71]. The fracture risk associated with exogenous glucocorticoids can only be

partially attributed to the reduction in bone mineral density, with patients with similar BMD but no glucocorticoid use suffering significantly fewer fractures [72, 73]. This suggests an impact of glucocorticoids on bone quality as well as density. It is important to note that following cessation of glucocorticoid therapy, the fracture risk gradually declines, and returns to background levels within a few years [71].

Glucocorticoids have both direct and indirect pathways contributing to bone loss, with glucocorticoids causing a reduction in intestinal calcium absorption, an increase in renal calcium clearance, and suppression of growth hormone and sex hormones (testosterone and oestrogen) [74]. All of these factors may result in increases in bone loss and interfere with bone metabolism, with an attenuation of linear growth removing the impetus for bone strength to be increased via the link to periosteal apposition. However, the main mechanisms by which glucocorticoids impact upon bone health is likely to be by their direct action on bone cells.

Pharmacological doses of glucocorticoids inhibits osteoblast differentiation and function, and also induces osteoblast apoptosis [75-77]. This results in a profound suppression of bone formation. High concentrations of glucocorticoids result in a down regulation of signalling pathways that promote osteoblastogenesis, namely Wnt/ β -catenin [78] and bone morphogenic protein-2 (BMP-2) signalling [79], and increases pro-apoptotic factors of the Bcl-2 family [76, 77]. Animal studies have also suggested that in the presence of excess glucocorticoids there is also an increase in expression of transcription factors that are crucial for adipocyte differentiation, resulting in increasing mesenchymal stem cell commitment to the adipocyte lineage, rather than differentiation into osteoblasts [79]. A reduction in osteoblastogenesis also results in a loss of osteoblast-generated proteins, such as collagen and osteocalcin [79], which further reduces bone integrity.

Excess glucocorticoids also impact upon osteocytes and osteoclasts. It has been described that osteocytes are the terminal differentiation product of osteoblasts, and are located in the lacunar-canalicular network of mineralised bone [80]. This is a fluid-storage system and also contains a vascular network allowing communication and nutritional support to the enclosed osteocyte population. In murine models high concentrations of glucocorticoids result in a reduction of intra-osseous vasculature and a reduction in solute transport from the circulation to the lacunar-canalicular network [81]. This will result in a reduction in bone strength and increased

bone fragility. The osteocyte network acts as a mechanosensor which maintains bone integrity by recruiting osteoclasts and osteoblasts as appropriate to sites of active bone remodelling in response to mechanical stimulation [82]. *In vivo* models have shown an increase in osteocyte autophagy in situations of glucocorticoid excess [83, 84]. This can result in an accumulation of autophagosomes which create a toxic environment for osteocytes [84]. Glucocorticoids also increase osteocyte apoptosis, which has been linked to activation of proapoptotic factors Pyk2 and JNK [85].

Osteoclasts are bone resorbing cells derived from the monocyte-macrophage lineage. A study of glucocorticoid treatment in patients with multiple sclerosis reported an initial increase in osteoclast activity and number [86]. However, with prolonged use of glucocorticoids there was a suppression in the proliferation of osteoclast precursors, as well as a block in osteoclast function [87].

It is clear that prolonged glucocorticoid use appears to have a role in ultimately suppressing both osteoclastic and osteoblastic function, with an overall reduction in bone turnover. Bone remodelling is crucial in removing ineffectual tissue and replacing it with new material. Disruption of this process is likely to be a cause of the poor bone quality experienced by patients receiving these drugs.

1.5 Background of current UK treatment for acute lymphoblastic leukaemia

The first case of leukaemia diagnosed by microscopic examination was described by Henry Fuller in 1850, but at that time the condition was universally fatal. In 1948 'temporary remission' induced by aminopterin, a folic acid antagonist, was described in 5 children with acute leukaemia [88], beginning the era of chemotherapy for ALL. In the past 60 years there has been significant progress in the treatment of childhood leukaemia, predominantly through increasing intensification, use of combination chemotherapy and a prolonged maintenance phase of chemotherapy. Outcomes for patients diagnosed with ALL have improved dramatically due to well-designed sequential clinical trials.

In the USA in 1961 a complete remission rate of 59% and a 2 year survival of 20% was described in 39 patients for whom a combination of mercaptopurine and methotrexate was used [89]. However, for the majority of patients ALL continued to be fatal, prompting the development of a multi-

component therapeutic approach for patients at St Jude's Hospital, USA in 1962 [90]. Since 1970 the UK Medical Research Council Working Party on Childhood Leukaemia has conducted a series of therapeutic trials for ALL and has shown a stepwise improvement in prognosis for children with ALL, from a 5 year Event Free Survival (EFS) of 35% in 1972 to 87% in 2010. Results of the early trials highlighted the importance of uninterrupted therapy, and sustained exposure to maximum tolerated doses of therapy [91, 92]. The most significant prognostic factors were found to be age, leukocyte count, gender [93], genetic factors and response to initial therapy, with routine fluorescence in situ hybridization (FISH) screening for high risk genetic abnormalities and risk stratification introduced in 1997 [91, 94].

In ALL 97 and ALL 97/99 there was randomisation of the efficacy of dexamethasone 6.5mg/m² for 28 days and prednisolone 40mg/m² for 28 days and for 5 days in monthly pulses during maintenance. It was found that there was a major improvement in central nervous system (CNS) relapse rate in ALL 97/99, with the rates nearly halved for both standard and high-risk patients (from 7% to 4%). This was regardless of type of steroid, but the best results were in patients who were randomised to dexamethasone, in whom the actuarial isolated CNS relapse rate was only 1.8% at 5 years, compared to 3.7% in those randomised to prednisolone [91].

By 2002 there was recognition that analysis of minimal residual disease (MRD) was the strongest predictor of outcome in children undergoing therapy [95], and in October 2003 UKALL 2003 opened. The results from UKALL 2003 provided further evidence of the benefit of treatment intensification to patients defined as high risk by MRD measured at day 29 of induction [96]. UKALL 2003 also included a randomised treatment change based on MRD at day 29, with low risk patients (undetectable MRD at the end of induction/week 11) randomly assigned to 1 or 2 courses of delayed intensification (DI). There was found to be no significant difference in EFS between the groups, with a reduction in relapse risk resulting in a 5 year EFS of 87%, with an overall survival of 91% [96].

1.6 Current treatment for acute lymphoblastic leukaemia

With the significant improvement in survival after treatment for ALL, there has been an increasing focus on reducing the toxicity of treatment. The majority of young people diagnosed with ALL or LBL between 26/04/2012 and 31/12/2018 consented to participate in the national trial, UKALL 2011

(ISRCTN64515327, Eudract 2010-020924-22). UKALL 2011 [97] was designed to improve survival and quality of survival by addressing:

- treatment related mortality and morbidity
- poor prognosis of CNS relapse
- poor prognosis of very early marrow relapse
- superior outcomes seen for young adults treated on paediatric protocols

The aim was to define whether further refinement of MRD based risk stratification and treatment regimen improves survival whilst reducing overall burden of therapy in children and young adults (age 1 to 24 years and 364 days) diagnosed with ALL or LBL (T-cell non-Hodgkin's Lymphoma (NHL) or Smlg-ve precursor B-NHL).

At the time of diagnosis patients with B cell precursor ALL (BCP ALL) are stratified into standard or high risk using the National Cancer Institute (NCI) risk stratification approach. Standard risk therapy is used for patients who are aged ≥ 1 year and < 10 years old at diagnosis and with a highest white cell count (WCC) before starting treatment of $< 50 \times 10^9/L$. Patients in this group receive a 3-drug induction, known as Regimen A. Patients aged ≥ 10 years at diagnosis and/ or with a diagnostic WCC of $\geq 50 \times 10^9/L$ receive a 4-drug induction, known as Regimen B. All patients with T cell ALL, or either B cell or T cell LBL, receive Regimen B induction, as do patients who have known high risk cytogenetics at the start of treatment. All patients with Down syndrome receive Regimen A induction. If patients have CNS disease at diagnosis they receive additional weekly intrathecal methotrexate until 2 consecutive clear samples of cerebrospinal fluid (CSF) are obtained. If CSF is clear by day 29 they continue with NCI and MRD directed therapy. If there is persistence of CNS disease the patient transfers to Regimen C with MRD measured at week 14. Those that remain at high risk were taken off protocol, as were patients who fail to respond adequately to induction therapy ($\geq 25\%$ of blasts at day 29 or T-ALL with MRD $> 10\%$).

As the focus of the latter parts of this dissertation is on the therapy and bone toxicity for patients over the age of 10 years at diagnosis of ALL or LBL, the emphasis will now be on this group of patients. Figure 5 illustrates the chemotherapy regimen for these patients.

Post induction treatment for patients over the age of 10 years is determined by MRD in ALL patients, or tumour volume assessment in patients with lymphoblastic lymphoma. Patients with no MRD results are assessed by morphology (% of blasts at day 8 of induction).

There were originally 2 randomisations within UKALL2011, the first in induction and the second in maintenance. The objective of the first randomisation was to reduce toxicity through the introduction of a short (14 day) course of high dose (10mg/m²/day) dexamethasone, rather than the standard 28 days of 6m/m²/day. The primary outcome measure of this randomisation was steroid induced morbidity and mortality, defined as all serious adverse events and grade 3 or 4 adverse events related to induction and categorised as steroid related or steroid contributory (including ON). The first randomisation was closed in March 2017 following an interim review of data with a formal futility analysis by the independent Data Monitoring Committee. This confirmed no clear benefit in administering a short course of dexamethasone in reducing adverse events, compared with adverse events experienced on standard dexamethasone.

The second randomisation was at the start of interim maintenance and investigated the effect on CNS relapse and quality of life in patients receiving either high dose methotrexate without prolonged intrathecal therapy or the current standard UK CNS-directed ALL therapy with protracted intrathecal therapy. It also aimed to assess the effect on bone marrow relapse risk and quality of life in patients receiving monthly pulses of vincristine and dexamethasone in maintenance therapy. The methotrexate and pulses randomisation had a factorial design, with patients being randomised to receive either high dose methotrexate or standard interim maintenance followed by a single DI and either maintenance with pulses or without pulses of vincristine and dexamethasone. If a patient was randomised to high dose methotrexate therapy, they will have subsequent intrathecal methotrexate in maintenance, but could be randomised to either pulses or no pulses. If they were randomised to either standard or Capizzi interim maintenance they were randomised to maintenance therapy with or without pulses, and all patients received intrathecal methotrexate.

Following the results of previous studies, all patients in UKALL2011 are given a single block of DI, and augmented therapy is limited to those who are not MRD low risk.

Treatment lasts 2 years from the start of interim maintenance for female patients, and 3 years from the start of interim maintenance for male patients.

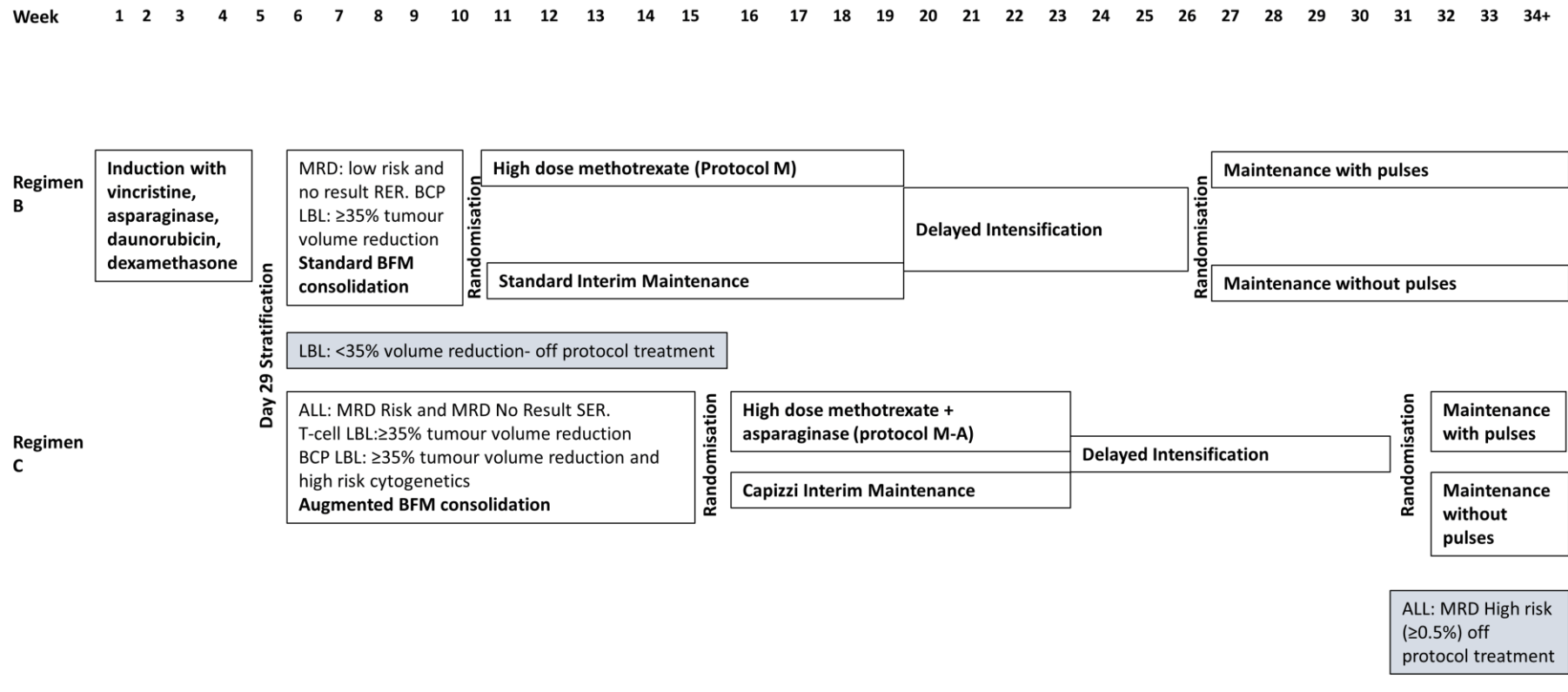
Details of all chemotherapeutic agents are provided in Appendix 1.

It should be noted that for patients who have a BMI >98th percentile the dosing of medication is calculated for a weight at the 98th percentile. For

children with a BMI <2nd percentile medication doses are calculated as for a patient with a weight at the 2nd percentile. There is also capping of doses for dexamethasone and vincristine as described in Appendix 1.

Patients who did not consent to participate in UKALL2011, or who were diagnosed after the trial closed (December 2018), receive the same treatment as those on the trial. At the point of randomisation they receive standard interim or Capizzi interim maintenance, depending on their risk stratification. At the next randomisation point they receive maintenance therapy with vincristine/dexamethasone pulses and intrathecal methotrexate.

Figure 5. UKALL 2011 trial schema for patients over 10 years of age



MRD: Minimal residual disease BFM: Berlin-Frankfurt-Munich

SER: Slow early response ($\geq 25\%$ blasts at day 8 of induction) RER: Rapid early response ($< 25\%$ blasts at day 8 of induction)

1.7 Glucocorticoid therapy in acute lymphoblastic leukaemia

Steroids have been used in the treatment of ALL in some studies since the 1970s, and both prednisolone and dexamethasone have been shown to be effective in improving outcome as a component of therapy [98]. However, the balance between efficacy and toxicity is critical, and numerous studies have looked at this relationship.

The CCG-1922 study randomised NCI standard risk patients to prednisolone versus dexamethasone during all phases of therapy except DI [99], and found a significant improvement in EFS for patients randomised to dexamethasone, with significantly lower CNS relapse. The UK Medical Research Council (MRC) ALL-97/99 study, open to standard and high risk patients, also randomised patients to dexamethasone (6.5mg/m²) or prednisolone (40mg/m²) except during DI, and also found an improved EFS, as well as improved risk of relapse in the dexamethasone group [100].

In contrast to these studies, where substitution of dexamethasone for prednisolone was at a ratio of 1:6, the use of higher relative doses of prednisolone (1:7.5 or 1:10) was found to negate the impact of dexamethasone on relapse [101, 102].

As dexamethasone became more commonly used, concerns about increases in treatment related mortality also increased. In the MRC ALL-97/99 study [100] major steroid related toxicities included behavioural problems, myopathy, osteopenia, weight gain and liver enlargement. There was a higher incidence of toxicities in the dexamethasone rather than the prednisolone group, and this was also seen in the AIEOP-BFM ALL-2000 trial [103], particularly in patients over 10 years of age. In the DFCl 91-01 study, which randomised between dexamethasone and prednisolone after induction therapy, significantly more patients receiving dexamethasone had infections compared to those receiving prednisolone, but with no difference in remission death rates between steroid groups [104].

There were specific concerns about an increased risk of ON in patients treated with dexamethasone, but there are conflicting results between centres [100, 105-107], and this will be covered in more detail in the literature review. The differences between centres may be due to differences in ascertainment, as well as the different patient populations and environments. Phase of exposure, and continuous versus split dosing of dexamethasone may also result in varying risk of ON. There are particular

challenges in understanding ON in patients with ALL, as ALL has the highest incidence in infants aged 1-4 years, and incidence drops sharply through childhood and adolescence. As ON predominantly affects older children and adolescents, who are less commonly diagnosed with ALL, side effects of treatment in this population are more challenging to identify and understand.

Although the clinical benefits of therapeutic glucocorticoids can hardly be overestimated, unwanted effects, such as a reduction in bone health [108] may be the price that is being paid.

Chapter 2 Literature review

This literature review consists of 3 main parts. The first part reviews the medical literature around the development of ON in young people with ALL. The second part of the chapter reviews the medical literature around BMD and fracture risk of young people with ALL. The final part reviews potential therapeutic interventions. For the purpose of this thesis, the focus is on the use of bisphosphonates for the management of ON in young people with ALL, and the role of vitamin D supplementation.

2.1 Osteonecrosis in young people with acute lymphoblastic leukaemia

2.1.1 Research questions

Within this review I aim to cover the following questions:

1. What is the prevalence and incidence of ON in children and young adults being treated for ALL at different time points in their treatment?
2. What are the current classification systems for ON?
3. What is the natural history of ON in young patients with ALL?
4. What are the risk factors for development and progression of ON in this population?

2.1.2 Search strategies

The literature identified within this review was achieved by a search using the following databases to identify original published studies: Medline 1946-2015, Embase 1996-2015, EBM databases, Journals @Ovid, Books@Ovid. In addition, I searched the reference lists of relevant studies.

There were no MeSH headings for 'osteonecrosis', 'avascular necrosis' or 'aseptic necrosis', so these were searched as keywords. Results were combined with AND 'leukaemia' as a MeSH term.

Duplicate references were manually removed and eligibility judgements were made on the basis of relevant clinical or disease information found in the article abstract and full article when appropriate.

Further searches included the terms 'steroids' or 'glucocorticoids' or 'dexamethasone' or 'prednisone' or 'prednisolone' AND 'leukaemia', but no new articles were found.

My initial search had a yield of 185 articles. Of these, 40 articles or abstracts were found to be relevant to my study questions. During the research period weekly reports from Ovid and Embase with any of the above terms in the abstract, title or as a keyword were reviewed and added, and was last updated in May 2019.

A summary of relevant studies are presented in tabulated format. Retrospective studies are presented in Table 1, prospective studies in Table 2 and genetic studies are presented in Table 3.

Table 1. Retrospective studies reporting prevalence and risk factors for symptomatic osteonecrosis in children and young adults with acute lymphoblastic leukaemia

First author, Country, Year of publication	Patient inclusion criteria for ALL study	Number of patients	Protocol (recruiting period) [type of steroid]	Study design and data source for diagnosis of ON	Follow-up (median, years)	Prevalence/ cumulative incidence	Timing of ON after diagnosis of ALL	Factors associated with ON	Factors not associated with ON
Arico Italy 2003[109]	Newly diagnosed non-B-ALL Age<18 years	1421	AIEOP ALL 95 (05/1995-12/1999) [prednisolone and dexamethasone]	Symptomatic ON assessed with supportive imaging. Onset of onset of ON reported in routine protocol data, ad hoc data recall on May 1, 2000. Evaluation of disease course on 1/04/2003	Median 3.2 years	Prevalence: 1.1%. Cum inc at 5 years: 1.6%	Median 17 months (range 8-45 months)	Age >10 years Gender F>M Risk Group High>standard>intermediate	
Badhiwala Canada 2015[110]	Newly diagnosed ALL Age2-18 years	208	DFCI protocols 91-01, 95-01, 00-01, 05-01 (01/1992-12/2010) [prednisolone and dexamethasone]	Case note study. Symptomatic ON confirmed by X-Ray, CT, MRI, or Technetium-99m bone scan		Prevalence: 10.1% (18.8% for protocol 05-01, 10.9% for 00-01, 4% for 95-01, 0% for 91-01)	Average 69.2 weeks after diagnosis of ALL.	Age ≥10yrs Post induction PEG-ASP Thromboemboli	Gender Risk category Post induction corticosteroid (dexamethasone vs prednisolone) BMI Cranial radiation

First author, Country, Year of publication	Patient inclusion criteria for ALL study	Number of patients	Protocol (recruiting period) [type of steroid]	Study design and data source for diagnosis of ON	Follow-up (median, years)	Prevalence/cumulative incidence	Timing of ON after diagnosis of ALL	Factors associated with ON	Factors not associated with ON
Burger Germany 2005[111]	Newly diagnosed non-B-ALL Age 0-18 years	1951	ALL-BFM 95 (01/01/1996-20/06/2000) [prednisolone and dexamethasone]	Questionnaire to multiple centres. All patients > 10 years of age specifically listed on questionnaire.	Not available	Cum inc at 5 years: 1.8% For age <10yr: 0.2%, age ≥10yrs: 8.9%, age ≥15yrs: 16.7%.	35% within first 12 months, 32% within second year, 29% within third year	Age ≥10 years Risk group: moderate or high risk	Gender
Chen Taiwan 2015[112]	Newly diagnosed ALL. Age<18 years	245	Taiwan Pediatric Oncology Group-ALL-2002 protocol. (01/2002-12/2011) [prednisolone and dexamethasone]	Symptomatic patients had X-ray, MRI or Tc-99m bone scan to diagnose ON using Ficat classification	4.7 years (range 2 weeks-8.7 years)	Prevalence: 2.4% Cum inc at 5 years: 2.2% Cum inc at 8 years: 3.4%	Median 2.5 years	Age > 10 years Gender F>M	
De Moerloose Belgium/France/Portugal 2010[113]	Newly diagnosed ALL or NHL Age <18 years	411	EORTC- 58951 (06/1999-11/2002) [dexamethasone or prednisolone]	Method of diagnosing ON not documented. Only grades 2 and 3 ON reported.	6	Prevalence: 3%		Vincristine and corticosteroid pulses in maintenance vs no pulses	

First author, Country, Year of publication	Patient inclusion criteria for ALL study	Number of patients	Protocol (recruiting period) [type of steroid]	Study design and data source for diagnosis of ON	Follow-up (median, years)	Prevalence/cumulative incidence	Timing of ON after diagnosis of ALL	Factors associated with ON	Factors not associated with ON
Elmantaser Scotland 2010[114]	Newly diagnosed ALL Age 1-<25 years (depending on protocol)	186	UKALL97, UKALL97/01, UKALL2003 (01/1997- 12/2007) [dexamethasone or prednisolone, depending on protocol]	Retrospective survey of case notes. Symptomatic ON confirmed by XR and MRI	5.7 years for boys, 5.9 years for girls	Prevalence: 9.7%	Median 29 months after start of chemotherapy	Age >9yrs Dexamethasone protocols (compared with prednisolone)	Gender
Heneghan USA 2016[115]	Newly diagnosed ALL	10,729	Not specified-varying depending on treatment centre (01/2004-07/2012)	Retrospective cohort study. Used Pediatric Health Information System database for screening for ON ICD-9 code. ON confirmed by review of XR and MRIs	5 years from first ALL admission	Cum inc at 5 years: 2.3%	Median 1.4 years, 35% within 1 st year, 31% in 2 nd year, 24% in 3 rd year, 5% in 4 th year, 5% in 5 th year	Age: >10 years	Race

First author, Country, Year of publication	Patient inclusion criteria for ALL study	Number of patients	Protocol (recruiting period) [type of steroid]	Study design and data source for diagnosis of ON	Follow-up (median, years)	Prevalence/cumulative incidence	Timing of ON after diagnosis of ALL	Factors associated with ON	Factors not associated with ON
Kadan Lottick USA 2008 [116]	Leukemia, CNS malignancy, Hodgkin's disease, non-Hodgkin's lymphoma, malignant kidney tumour, neuroblastoma, soft tissue sarcoma, or bone tumour	2697 patients with ALL.	Multiple treatment protocols due to varying pathologies (1970-1986) [not documented]	Childhood Cancer Survival Study. Patient questionnaires: ON diagnosis by patient recall	Not available	Prevalence: 0.2% for patients <10 years at diagnosis, 2.8% for patients ≥16 years of age	35% had diagnosis between 0-4 years, 31% between 5-14 years, 35% after 15 years	Age ≥16 years Radiation therapy Dexamethasone (compared with prednisolone) Stem cell transplantation alkylator history Methotrexate history	Pituitary radiation Gender Race (white vs non-white) BMI
Karol USA 2015[117]	Discovery cohort: Newly diagnosed high risk B-ALL	2285	Discovery cohort: COG AALL0232 protocol. (recruiting period not documented)	MRI of symptomatic patients in discovery cohort	Not available	Prevalence: 10.9%	Not documented	Age≥10 years Gender F>M Ethnicity European>Africa n Asparaginase exposure	
Korholz Germany 1998[118]	Newly diagnosed ALL. Age 1-17 years	121	CoALL 3-85, 4-89, 5-92 and BFM-ALL 86 and 90 (1986-1992) [dexamethasone]	MRI of symptomatic patients	Not available	Prevalence: 8%	Median 17.5 months	Age ≥10 years HR ALL	WCC at diagnosis

First author, Country, Year of publication	Patient inclusion criteria for ALL study	Number of patients	Protocol (recruiting period) [type of steroid]	Study design and data source for diagnosis of ON	Follow-up (median, years)	Prevalence/cumulative incidence	Timing of ON after diagnosis of ALL	Factors associated with ON	Factors not associated with ON
Mattano USA 2000[119]	Newly diagnosed high risk ALL Age 1-20 years	1409	CCG-1882 (05/1989-06/1995) [prednisolone and dexamethasone]	Symptomatic ON diagnosed with varying imaging techniques. Survey Mar 1996, with follow up questionnaire. Additional data from CCG-1882 database and patient data records	Not available	Cum inc at 3 years: 9.3% <10 years 0.9% 10-15 years 13.5% 16-20 years 18%	32% during 1 st year, 54% in 2 nd year, 13% in 3 rd year.	Age≥10 years Gender F>M Ethnicity whites>other>blacks	Single or double DI
Mogensen Denmark 2018[120]	Newly diagnosed ALL Age 1-45 years	1489	NOPHO ALL2008 (2008-2014) [dexamethasone or prednisolone in induction, dexamethasone in DI]	Symptomatic ON prospectively registered via toxicity registry. ON diagnosis verified by MRI or radiographs in local treatment centres	Not available	Cum inc at 5 years: 6.3%	Median 1.4 years	Age 10-19 years Gender F>M (if aged 10-19 years)	ALL risk group Induction therapy Immuno-phenotype WCC BMI

First author, Country, Year of publication	Patient inclusion criteria for ALL study	Number of patients	Protocol (recruiting period) [type of steroid]	Study design and data source for diagnosis of ON	Follow-up (median, years)	Prevalence/cumulative incidence	Timing of ON after diagnosis of ALL	Factors associated with ON	Factors not associated with ON
Nachman USA 1998[95]	Newly diagnosed high risk ALL. Age range not specified	311	CCG-1882 A-BFM or CCG-BFM (01/1991-06/1995) [dexamethasone and prednisolone]	No information about method of identifying patients with ON or method of confirming diagnosis	Median 49 months	Cum inc at 3 years: 15.1% for augmented therapy group, 11.9% for the standard therapy group	Not documented		
Padhye Australia 2016[121]	Newly diagnosed ALL or lymphoblastic lymphoma Age range not specified	251	ANZCHOG study 8 (2002-2011) [prednisolone and dexamethasone]	Symptomatic ON, confirmed by MRI. Retrospective chart review.		Prevalence: 7%	Median 1.15yrs (range 0.25-2.12)	Age >10 years	T or B ALL
Parasole Italy 2018[122]	Newly diagnosed ALL Age 1-17 years	3691	AIEOP-BFM-ALL-2000 or AIEOP-ALL-R2006 (2000-2011) [dexamethasone or prednisolone]	ON confirmed by CT/MRI.	Not available	Prevalence: 2.7%	10% during maintenance 48% during maintenance 16% after EOT	Age >10 years	Steroid type in induction WCC Immunophenotype Risk group

First author, Country, Year of publication	Patient inclusion criteria for ALL study	Number of patients	Protocol (recruiting period) [type of steroid]	Study design and data source for diagnosis of ON	Follow-up (median, years)	Prevalence/ cumulative incidence	Timing of ON after diagnosis of ALL	Factors associated with ON	Factors not associated with ON
Patel UK 2008[123]	Newly diagnosed ALL Age 15-55 years)	1088 (age 15-55yrs), 155 age <20yrs,	UKALL XII/ECOG2993 (1993-2004) [prednisolone and dexamethasone]	Symptomatic ON identified by review of annual follow up forms and questionnaires. No criteria for diagnosis of ON	6.3 years	Cum inc at 3 years: Age <20yr: 12% at 3 years, 17% at 10 years Age >20 yr: 3% at 3 and 10 years	Median time 2.2 years from diagnosis	Age <20 years, Treatment type chemotherapy alone increased risk compared with allo-SCT or auto SCT	Gender
Sakamoto Japan 2018[124]	Newly diagnosed ALL Age 1-18 years	1162	JACLS ALL-97 and ALL-02 (1997-2008) [dexamethasone and prednisolone]	JACLS ALL-97: prospective reporting of symptomatic ON. ALL-02 retrospective data collection of symptomatic ON. Diagnosis confirmed on X-ray/ MRI/ CT	Not available	Cum inc at 5 years: JACLS ALL-97: 1.8% (CI 1.0-3.2%) JACLS ALL-02: 1.2% (CI 0.7-2.2%)	58.3% during chemotherapy, 41.7% after chemotherapy	Age ≥10 yr	Sex WCC at diagnosis Dexamethasone dose Immunophenotype Treatment with or without L-asparaginase
Salem Germany 2013[125]	Newly diagnosed ALL, AML or non-Hodgkin's lymphoma Age<18 years	80 with ALL	ALL-BFM, NHL-BFM, EICNHL-ALCL, GPOH-AML (1990-2010) [dexamethasone or prednisolone]	Diagnosis suspected on clinical features, subsequent radiographic imaging (X-rays/CT/MRI/bone scans) to confirm diagnosis.	5.7	Prevalence: 7.5%	Mean timing of ON 16.8	Age Patients mean 6.2 years older than mean age of cohort. Cumulative steroid dose	Gender

First author, Country, Year of publication	Patient inclusion criteria for ALL study	Number of patients	Protocol (recruiting period) [type of steroid]	Study design and data source for diagnosis of ON	Follow-up (median, years)	Prevalence/cumulative incidence	Timing of ON after diagnosis of ALL	Factors associated with ON	Factors not associated with ON
Sawicka Poland 2006[126]	Newly diagnosed ALL and NHL	191 (150 with ALL, 41 with NHL)	ALL BFM 95, BFM ALL IC 2002, New York for high risk patients (1999-2005) [prednisolone and dexamethasone]	Symptomatic patients. No information about how patients with ON identified	Not available	Prevalence: 4.1% of patients with ALL	20 months	Gender M>F	
Strauss USA 2001[127]	Newly diagnosed ALL. Age 0-18 years	176	DFCI 87-01/91-01 (11/1987-12/1995)	Case note review for bony morbidity. Symptomatic ON confirmed by at least one imaging study	Median 7.6 years	Prevalence: 7%	14 months	Age 9-18yrs	Risk group Gender WCC at diagnosis Dexamethasone vs prednisolone in post-remission therapy
Vora UK 2013[128]	Newly diagnosed ALL. Age 1-24 years.	3126	UKALL2003 (01/10/2003-30/06/2011) [dexamethasone]	Clinical reporting of symptomatic ON using toxicity reporting form/significant adverse event reporting form	4.75	Prevalence: 4%	Not documented	Age >10years Risk group Clinical intermediate risk vs standard risk	High risk vs Standard/intermediate risk

CI: Confidence Intervals

Cum inc: cumulative incidence

Table 2. Prospective studies reporting prevalence and risk factors for symptomatic osteonecrosis in children and young adults with acute lymphoblastic leukaemia

First author, Country, Year of publication	Patient inclusion criteria	Number of patients	Protocol (Recruiting period) [Type of steroid]	Study design and data source for diagnosis of ON	Follow-up (median, years)	ON frequency/ cumulative incidence	Timing of ON (from diagnosis of ALL)	Factors associated with ON	Factors not associated with ON
Ali Egypt 2018 [129]	Newly diagnosed ALL. Age 1-18 years.	665	Total therapy XV (01/2009-12/2012) [prednisolone and dexamethasone]	MRI screening of hips and knees at approximately 6.5 and 9 months from diagnosis, at completion of chemotherapy, or any time if symptomatic	Not documented	Cum inc at 5 years: 11.96%	Mean 21 months	Age >10 years Risk group HR and IR	Sex
Den Hoed Netherlands 2015 [130]	Newly diagnosed ALL. Age 4-18 years	466	DCOG-ALL9 (01/1997- 11/2004) [Dexamethasone]	Prospective data collection. Symptomatic ON confirmed by MRI imaging	1 year after cessation of treatment (3 years)	Cum inc at 3 years: 6.4%	Median time 14 months (range 1-33 months)	Age BMD at cessation of treatment BMD one year after cessation of treatment	Gender Immunophenotype (BCP-ALL, T-ALL) Risk group Clinically significant fractures during treatment BMD at baseline
Kaste USA 2015 [131]	Newly diagnosed ALL aged 1-18 years	462	Total therapy XV (06/2000-10/2007) [Prednisolone and dexamethasone]	MRI screening of hips at approximately 6.5 and 9 months from diagnosis, and at completion of chemotherapy. Extensive femoral head ON: lesions affecting ≥30% of epiphyseal surface	Duration of therapy	Cum inc at 1 year: 17.1%, cum inc after completion of chemotherapy: 21.7%, extensive femoral head ON in 6.5%	Not applicable	Age >10 years at diagnosis Treatment regime high risk arm Race other than black, Hispanic or white	Gender BMI at diagnosis Physiologic patency

First author, Country, Year of publication	Patient inclusion criteria	Number of patients	Protocol (Recruiting period) [Type of steroid]	Study design and data source for diagnosis of ON	Follow-up (median, years)	ON frequency/ cumulative incidence	Timing of ON (from diagnosis of ALL)	Factors associated with ON	Factors not associated with ON
Kawedia USA 2010 [132]	Newly diagnosed ALL aged 1-18 years	364	Total Therapy XV (not documented) [Prednisolone and dexamethasone]	MRI screening of hips and knees at approximately 6.5 and 9 months from diagnosis, and at completion of therapy.	Duration of therapy	Cum inc at first screen: Grade 1: 38.7%, Grade 2-4: 2.2% Cum inc at one year from start of therapy: Grade 1: 35.4%, Grade 2-4: 14.6% Cum inc at completion of therapy: Grade 1: 53.9%, Grade 2-4 17.6%	Not applicable	-Asymptomatic ON: SR/HR treatment arm -Symptomatic ON: Age: >10yr SR/HR treatment arm high cholesterol wk 8 low serum albumin(wk 7) -Grade 3 or 4 ON: Age: >10yr SR/HR treatment arm High dexamethasone AUC wk 8 low serum albumin wk 8	-Asymptomatic ON: age (<10 vs >10 gender race (white vs non white) -Symptomatic ON: Race (white vs non white) Gender cortisol, cholesterol level at day 15, wk 2-5, wk 7, albumin wk 8, dexamethasone AUC at wk 7/8, BMI wk 7/8, triglycerides
Krull Germany 2018 [133]	Newly diagnosed ALL or LBL aged 10-17 years	76	AIEOP-BFM 2009 or COALL-08-09 NHL-BFM 2012	MRI screening of hips and knees at time of ALL LBL diagnosis and at 6 months	Not documented	Prevalence: 9.2% at diagnosis	Not applicable		
Mattano USA 2012 [134]	Newly diagnosed high risk ALL aged 1-21 years	2056	CCG-1961 (09/1996-05/2002) [Prednisolone and dexamethasone]	Prospective clinical monitoring for symptomatic ON, with imaging of suspected sites (imaging modality as per local policy).	7.8	Cum inc at 5 years: 7.7±0.9%	41% in 1 st year of diagnosis, 47% in 2 nd year, 9% in 3 rd year, 3% in 4 th year	Age > 10 years. Gender F>M Continuous versus alternate week dex (age 10+)	Rapid/slow responders Baseline laboratory or ALL characteristics. Standard or intensified post induction therapy

First author, Country, Year of publication	Patient inclusion criteria	Number of patients	Protocol (Recruiting period) [Type of steroid]	Study design and data source for diagnosis of ON	Follow-up (median, years)	ON frequency/ cumulative incidence	Timing of ON (from diagnosis of ALL)	Factors associated with ON	Factors not associated with ON
Mitchell UK 2005 [100]	Newly diagnosed ALL aged 1-18 years	1603	ALL97, ALL97/99 (04/1997-2002) [Dexamethasone or prednisolone]	Prospective reporting of symptomatic ON, grades 3+4. No documentation of method of ON diagnosis	4 years 11 months	Prevalence of grade 3 or 4 ON: 0.9%	Not available	Age specific high risk ages not specified but ON associated with older children Sex F>M	Dexamethasone vs prednisolone
Mogensen Denmark, Sweden, Finland, Norway, Iceland 2018 [135]	Newly diagnosed ALL aged 1-45 years	1489	NOPHO ALL2008 (07/2008-12/2014) [dexamethasone or prednisolone during induction, dexamethasone in DI]	Prospective reporting of symptomatic ON, diagnosis verified by MRI or radiographs	Not documented	Cum inc at 5 years: 6.3% (CI 4.9-8.0)	1.4 years	Age 10-18.9 years Sex F>M	Risk group Induction with prednisolone or dexamethasone Immunophenotype White blood cell count BMI Pubertal development
Niinimäki Finland 2007 [136]	Patients in complete remission from ALL at end of treatment. Aged 1-16 years	97	Nordic ALL protocols. (09/1992-12/2005) [HR and IR received prednisolone and dexamethasone, SR treated on 86 or 92 protocol received prednisolone only]	Lower limb MRI screening at cessation of therapy	Not applicable	Prevalence: 24% Grade 1-4 ON 7% symptomatic ON	Not applicable	Higher dexamethasone dose (BMI>95 th percentile at end of treatment Sex F>M Age	Prednisolone equivalent dose, methotrexate dose
Ojala Finland 1997 [137]	Children who had completed treatment for ALL	28	SR NOPHO, IR NOPHO, HR NOPHO (05/1992-04/1996) [Prednisolone with dexamethasone in IR and HR protocols]	MRI screening of extremities at cessation of therapy	Not applicable	Prevalence: 32% asymptomatic ON (CI 16-52%), 14% symptomatic	Not applicable	Not applicable	Not applicable

First author, Country, Year of publication	Patient inclusion criteria	Number of patients	Protocol (Recruiting period) [Type of steroid]	Study design and data source for diagnosis of ON	Follow-up (median, years)	ON frequency/ cumulative incidence	Timing of ON (from diagnosis of ALL)	Factors associated with ON	Factors not associated with ON
Ojala Finland 1998 [138]	Children 5 years post treatment for ALL	25	Nordic SR-86, BFM IR-83, BFM HR-86 (03/1995-11/1996) [Prednisolone with dexamethasone in IR and HR protocols]	MRI screening of lower extremities 5 years after cessation of therapy	Not applicable	Prevalence: 8% asymptomatic ON (all patients treated with HR protocol). No patients symptomatic	Not applicable		
Ojala Finland 1999 [139]	Newly diagnosed ALL aged 1-16 years	24	NORDIC-SR-92, BFM IR-83, NORDIC IR-92, NORDIC HR (10/1991-05/1996) [Prednisolone and dexamethasone]	Lower limb MRI screening at beginning of therapy, during therapy and at cessation of therapy.	Not applicable	Cum inc at cessation of therapy 38% (12% symptomatic)	12 months median interval from initiation of treatment to diagnosis of ON. (range 8-25 months)		Sex Primary leucocyte count
Ribeiro USA 2001 [140]	ALL or advanced stage NHL aged <18 years	107 with ALL, 116 in total	Total Therapy XIII A for ALL patients, NHL XIII for NHL patients (12/1991-08/1994) [Prednisolone. Dexamethasone for relapsed ALL]	MRI screening of hips and knees after at least one year of therapy. Further MRI every 6 months if still undergoing treatment or if evidence of ON (until lesions improved or stabilised)	Not available	Prevalence: 15.5% (grades 1-4), 9.5% symptomatic. 14% of patients with ALL had evidence of ON, 8.4% symptomatic	Median time 3 years from diagnosis (range 1yr- 5.6yrs)	Age >10yr	WBC BMI Sex Cumulative steroid dose, Cumulative methotrexate dose Dexamethasone treatment

First author, Country, Year of publication	Patient inclusion criteria	Number of patients	Protocol (Recruiting period) [Type of steroid]	Study design and data source for diagnosis of ON	Follow-up (median, years)	ON frequency/ cumulative incidence	Timing of ON (from diagnosis of ALL)	Factors associated with ON	Factors not associated with ON
Te Winkel Canada 2011 [141]	Newly diagnosed ALL aged 1-18 years	694	DCOG-ALL9 (01/1997-11/2004) [Dexamethasone]	Prospective study of symptomatic ON, confirmed by MRI imaging	1 year after cessation of treatment (3 years)	Cum inc at 3 years: 6.1%	Mean 1.2 years from diagnosis, range 0.1-2.7 years. 2.6% in induction, 2.6% intensification 87% in maintenance phase. 7.9% after cessation of therapy	Age older age Gender F>M	BMI at diagnosis Risk group
Toft Denmark, Sweden, Lithuania, Iceland, Estonia, Norway, Finland 2015 [142]	Newly diagnosed ALL aged 1-45 years	1076	NOPHO ALL2008 (07/2008-04/2013) [Prednisolone and dexamethasone]	Prospective toxicity reporting (3 monthly reporting) of symptomatic ON. Method of diagnosis of ON not reported	3.3 years	Prevalence: 3.4%	Not available	Age highest risk in age 10-14 years Risk group HR group lower incidence of ON	Sex
Mogensen Denmark 2017 [143]	Newly diagnosed ALL Aged 5-45 years	112	NOPHO ALL2008 (start not described, end 08/2015) [prednisolone and dexamethasone]	Prospective reporting of symptomatic ON	Not available	Prevalence: 22.9%	Not available	Hyperlipidaemia-peak triglycerides and total cholesterol	Age Sex Risk group White blood cell count Steroid during induction BMI

CI: Confidence Intervals

Cum inc: cumulative incidence

Table 3. Main studies assessing genetic risk factors for development of osteonecrosis in children and young adults with acute lymphoblastic leukaemia

First author, Country, Year of publication	Patient inclusion criteria	Number of patients	Protocol (recruiting period) [type of steroid]	Risk factors identified associated with ON	Factors not associated
French USA 2008 [144]	ALL and age>10 years	361	CCG 1882 (1989-1995) [prednisolone and dexamethasone]	PAI-1(SERPINE1) (OR 2.89, CI 1.45-5.34)	VDR, TYMS, MTHFR, ESR, LRP5, BGLAP, ACP5, ABCB1, PTH, PHTR
Karol USA 2015[117]	Newly diagnosed ALL with high risk B-ALL	2285	COG AALL0232 protocol. (recruiting period not documented)	SNP rs10989692 Glutamate pathway	
Karol USA 2015 [145]	Newly diagnosed standard risk B-ALL age<10 years	Discovery cohort: 82 cases of ON, 287 controls.	Discovery cohort- COG standard risk ALL protocol AALL0331 (30/06/2012 onwards. End date not documented)	BMP7 PROX1-AS1 variants.	
Relling USA 2004[146]	ALL with bilateral hip MRI performed	64	Total XIII B or XIV (1994-1999) [prednisolone and dexamethasone]	TYMS 2/2 genotype (OR 7.2, CI 1.05-48.9) and VDR FokI CC genotype (OR 3.7, CI 0.97-14.1).	CYP3A4*1B, CYP3A5*3, MDR1 exon 26, MDR1 exon 21, RFC, MTHFR C677T, VDR Fok I, VDR intron 8/exon 9, UGT1A1*28, GSTM1, MTHFR A1298C, NR3C1, GSTP1, TMPT, GSTT1
Plesa Canada 2017 [147]	Caucasian children with ALL	304	DFCI 87-01, 91-01, 95-01, 00-01 (01/87-07/05) [prednisolone in induction, dexamethasone depending on protocol]	BCL2L11 gene: polymorphisms 29201C>T, 891T>G	

2.1.3 Prevalence and incidence of osteonecrosis in children and young adults being treated for acute lymphoblastic leukaemia at different time points in their treatment

It is important to recognise from the outset that amongst the studies there is significant variation in reporting of ON, with a range of methods used for diagnosis of ON and potentially varying indices of suspicion. When assessing prevalence and incidence of ON, the majority of studies provide results for prevalence, but provide insufficient information about follow up time to allow an incidence rate to be calculated. Only a few studies provide results for cumulative incidence of ON, which gives additional information about timing of lesions. From Tables 1-3 it can be seen that the reported prevalence and cumulative incidence of ON in patients treated for ALL varies considerably, depending on study type, patient population and method used for diagnosis of ON.

The cumulative incidence of ON is unsurprisingly much lower in retrospective studies compared with prospective studies, with 5 year cumulative incidence ranging from 1-15% in retrospective reports [109, 111, 112, 115, 123, 148, 149], compared with a cumulative incidence of up to 53.9% in prospective studies where asymptomatic lesions are also reported [132]. The cumulative incidence of ON is higher in studies evaluating only high risk patients [105, 119, 134], and higher rates of symptomatic ON have been consistently reported in patients over 10 years of age at diagnosis of ALL [95, 119, 150]. In one retrospective study of high risk patients reported by Mattano et al, the 3 year cumulative incidence for symptomatic ON was 9.3%, which rose to 18% when only those aged 16-20 years were assessed [119].

A lower incidence of ON was reported in older studies with no planned reporting of ON [116]. This may be due to reduced clinician awareness of ON, combined with less steroid intensive therapeutic regimens. In the retrospective studies assessed there was considerable variability in method of diagnosing ON. Some studies based the diagnosis on symptom report, whilst others required imaging, using either plain X-Rays, CT, MRI or Technetium-99m bone scans. When retrospective questionnaires were used there is likely to be under-reporting of ON as only the more severe cases may be recalled, particularly if there is a significant delay between treatment and survey.

Prospective studies with MRI screening are more reliable in the reporting of ON, and are essential for the diagnosis of asymptomatic ON.

In the largest study with prospective MRI screening to assess both symptomatic and asymptomatic ON of the hip, the cumulative incidence of ON involving at least one hip was 17.1% after 1 year, and 21.7% after completion of therapy (4 years) [131]. By the end of therapy, extensive femoral head ON affecting $\geq 30\%$ of the epiphyseal surface had developed in 6.5% of all patients, and in 24% of those aged over 10 years [131]. An earlier report of this study by the St Jude Children's Research Hospital describes results of MRI screening in both hips and knees [132]. They found that at one year of therapy, the cumulative incidence for symptomatic ON was 14.6%, with an incidence of 35.4% for grade 1 ON (asymptomatic ON). At the end of therapy the cumulative incidence for symptomatic ON was 17.6%, with a cumulative incidence of 53.9% for asymptomatic ON [132]. Interestingly, when the same protocol was used for an Egyptian population, with prospective MRI screening at the same time points the reported 5 year cumulative incidence of symptomatic and asymptomatic ON was only 12%, with 38% of those asymptomatic [129]. However, this report was less comprehensive, with no information about median length of follow up or incidence of ON at each screening visit.

When assessing the timing of development of ON lesions, study results suggest that the majority of patients who develop symptomatic ON do so within the first 3 years of treatment [109-111, 115, 119, 127, 150], although a number of these studies had a relatively short follow up period, potentially missing patients who later developed ON. A large prospective study assessing symptomatic ON, with a median follow up of 7.8 years, found that only 3% of patients who developed symptomatic ON did so after the 3rd year after diagnosis of ALL, with no patients developing ON after the 4th year [134]. This study found 41% of patients who developed symptomatic ON did so within the first year of diagnosis, with 47% developing it in the 2nd year. Cross sectional studies suggest that in ALL ON often resolves 5 years after cessation of therapy, with the prevalence of asymptomatic ON falling from 32% at the end of treatment to 8% 5 years after cessation of therapy [137, 138]. The prevalence of symptomatic ON also fell, from 14% to 0%, although conclusions drawn from these studies must be limited by consideration of study size [137, 138].

For asymptomatic ON, the prospective studies suggest that the bone lesions develop early in the course of ALL therapy, with 38.7% of patients having Grade 1 ON by approximately 6.5 months after start of ALL treatment [132].

A recent study with early MRI screening for ON performed screening MRI scans at a median of 12.5 days (range 1-70 days) after ALL/LBL diagnosis [151]. It was found that of the 76 patients, 7 (9.2%) presented with an osteonecrotic lesion, with an average of 2 joints/patient affected. Of note, this study found that leukaemic infiltration of bone at diagnosis was not associated with osteonecrotic lesions [151].

In applying these results when considering management strategies for patients it is important to recognise that the majority of studies were not based in the UK. The population demographics and differences in treatment protocols may therefore make the results of these studies less applicable to our patient population.

The only UK study with prospective MRI screening was a pilot study, with results presented in an abstract [152]. This identified features of ON in 62% of patients when they had an MRI scan of hips, knees and pelvis at the start of maintenance chemotherapy [152]. This study looked only at patients over 9 years of age, and hence the prevalence is higher than would be expected if all paediatric patients with ALL were to be screened. Prior to the analysis described in this thesis, the largest retrospective UK study found a prevalence of symptomatic ON of 4% [128] but there was no information about how or when ON was diagnosed.

2.1.4 Current classification systems for osteonecrosis

Any classification system of musculoskeletal conditions should be consistent, logical, reproducible, all-inclusive, sensitive and clinically useful. It should organise and categorise a problem to guide decision making, and should stratify the natural progression or resolution of disease process [153]. A successful classification system should also be both reliable and valid. Reliability reflects the precision of a classification system, indicated by inter-observer reliability. Validity reflects the accuracy with which the classification system describes the true pathological process. To quantify validity the classification system must be compared with a gold standard, which is often difficult to establish due to observer bias [153]. Because of the difficulties with establishing validity, it is crucial that classification systems have a high degree of reliability. The kappa value is a measure to assess agreement between observers occurring above and beyond chance alone [154], and is the most accepted method of measuring observer agreement for categorical data. Kappa values range from -1 (complete disagreement) to 1 (complete agreement).

Most classification systems for ON are joint specific, and few have been validated for children and young people with ALL. The most common joints affected by ON in children and young people with ALL are hips, knees and ankles [155], but ON in other joints is also well recognised. Most widely used classification systems were developed for adults with ON affecting the femoral head, and there is no universally accepted classification method for assessing severity and prognosticating about the condition.

One of the first classification systems for ON of the femoral head, before the advent of routine clinical MR imaging, was described by Ficat and Arlet [156]. Subsequent modifications allowed inclusion of symptoms and MRI findings. The modified Ficat and Arlet classification system is currently the most commonly used classification system for ON of the femoral head in the literature [157] and most surgeons use the four-tiered method (Table 4).

Table 4. Modified classification system of Ficat and Arlet

Stage	Radiographic findings
1	None (only evidence of ON on MR images)
2	Diffuse sclerosis, cysts (visualised on radiographs)
3	Subchondral fracture (crescent sign; with or without head collapse)
4	Femoral head collapse, acetabular involvement, and joint destruction (osteoarthritis)

There are a number of limitations to this model of classification. The original classification system did not use MRI, and required invasive techniques such as core decompression. Further modifications amended these issues, but when the modified classification system was assessed to determine inter and intra-observer reliability it was found that the mean inter-observer kappa reliability coefficient was only 0.46 with a mean kappa value of intra-observer reproducibility of 0.59 [158], indicating a lack of reliability. The other major limitation is that the system did not allow quantification of size of lesion, making subtle degrees of progression difficult to assess.

The second most commonly used classification system for ON of the femoral head is the University of Pennsylvania staging system [159], and this was the first major classification system to incorporate size of lesion. It is described in Table 5 and this classification system defines 7 stages using radiographs or MRI to stratify lesion size.

Table 5. Classification system of the University of Pennsylvania

Stage	Radiographic findings
0	Normal radiograph, bone scan and MR images
1	Normal radiograph. Abnormal bone scan/MR images. Subcategories: A: Mild (<15% of femoral head affected) B: Moderate (15-30% of femoral head affected) C: Severe (>30% of femoral head affected)
2	Cystic and sclerotic changes in femoral head A: Mild (<15% of femoral head affected) B: Moderate (15-30% of femoral head affected) C: Severe (>30% of femoral head affected)
3	Flattening of femoral head A: Mild (<15% of articular surface) B: Moderate (15-30% of articular surface) C: Severe (>30% of articular surface)
4	Flattening of femoral head A: Mild (<15% of surface and <2mm of depression) B: Moderate (15-30% of surface or 2-4mm of depression) C: Severe (>30% of surface or >4mm of depression)
5	Joint narrowing or acetabular changes Grade average of femoral head involvement, as determined in stage 4, and estimated acetabular involvement. A: Mild B: Moderate C: Severe
6	Advanced degenerative change

One study assessed the classification system of the University of Pennsylvania to determine inter and intra-observer reliability [160]. Sixty five hip radiographs with confirmed ON were reviewed by 6 clinicians, including surgeons and radiologists. Stage specific kappa values for inter-observer variation were lowest for stage 3 (kappa value 0.21) and highest for stage 6 (kappa value 0.80). For intra-observer variation, kappa values were lowest for stage 5 (kappa value 0.27) and highest for stage 6 (0.78).

The presence of the crescent sign in stage 3 and joint space narrowing in stage 5 markedly diminished the overall reliability of the classification system, with 30% of intra-observer errors involving stage 3. Intra-observer kappa values for stage 3 and 4 were 0.46 and 0.59 respectively.

A further attempt to develop a classification system for ON of the femoral head occurred after a meeting of the Association Research Circulation Osseous (ARCO) in 1991 [161]. The system is based on the University of Pennsylvania staging system and is the third most commonly used classification system [157]. The ARCO system that is most commonly used is described in Table 6.

Table 6. Association Research Circulation Osseous classification system

Stage	Radiographic findings	Techniques	Sub-classification	Quantitation
0	None	Radiography, CT, scintigraphy, MR imaging	n/a	n/a
1	Normal radiographs/CT. At least one other technique positive.	Scintigraphy, MR imaging	Location of lesion: Medial Central Lateral	Area of involvement : A: <15% B: 15-30% C: >30% Length of crescent: A: <15% B: 15-30% C: >30% Surface collapse and dome depression: A: <15% and <2mm B: 15-30% and 2-4mm C: >30% and >4mm
2	Sclerosis, osteolysis, focal porosis	Radiography, CT, scintigraphy, MR imaging	As stage 1	As stage 1
3	Crescent sign and/or flattening of articular surface	Radiography and CT	As stage 1	As stage 1
4	Osteoarthritis, acetabular changes, joint destruction	Radiographs only	n/a	n/a

An additional classification system was developed by the Japanese Orthopaedic Association, which included location of lesion to aid stratification of ON [162], and is described in Table 7.

Table 7. Japanese Investigation Committee classification

Stage	Radiographic findings
Type 1A	Demarcation line appears in the femoral head The outer end of the demarcation line is located at the medial one third of the weight-bearing surface
Type 1 B	The outer end of demarcation line is located at the middle one third of the weight-bearing surface
Type 1C	The outer end of demarcation line is located at the lateral one third or more of the weight-bearing surface
Type 2	Shows early flattening of the weight-bearing surface, but does not reveal demarcation line
Type 3	Has cystic radiolucent lesion without demarcation line surface

This system was the basis of the MRI classification system by Sugano et al categorising necrotic lesions into Types A-C. Classification of the lesions is based on location as demarcated by a low intensity band on the central coronal plane of T1 weighted image [163]. Type A lesions occupy the medial one third or less of the weight bearing portion, Type B the medial two thirds or less and Type C greater than two thirds of the weight bearing portion. This simple classification system aimed to aid prognostication, with hips with type C lesions having a higher incidence of progression to collapse. However these systems assume lesions start medially and extend and there is no method of classifying lesions in which the acetabulum has become involved.

The importance of MR imaging was described in an article correlating MR results with radiographs [164]. MR images can distinctly demarcate ischaemic bone from normal bone within the femoral head. It was found that the agreement between plain radiographs and MR imaging was 80.6% for staging the disease, 71.2% for recording the location of the osteonecrotic lesion, 67.1% for evaluating the size of the lesion, 79.2% for the presence of collapse of the articular surface and 56.3% for the degree of collapse [164]. This highlights the value of MR imaging in the classification of ON, and indicates that the above classification systems could miss important information without MR imaging that could alter clinical management.

One of the few classification systems specifically designed for children with leukaemia and lymphoma was described by investigators at St Jude Children's Research Hospital (SJCRH), Memphis, USA, who developed a system specifically for diagnosis and grading of ON affecting the knee [165, 166] (Table 8).

Table 8. St Jude Children's Hospital categorisation for osteonecrosis

Category	Radiographic findings
1	osteonecrotic lesion absent
2	osteonecrotic lesion present but not extending to the articular surface
3	osteonecrotic lesion involving less than 25% of the articular surface
4	osteonecrotic lesion involving more than 50% of the articular surface
5	osteonecrotic lesion involving more than 50% of the articular surface

This categorisation system was developed specifically for use with MRI, and had a kappa value of 0.66 (CI 0.58-0.75) in locations where observers only had to record presence or absence of a lesion, and a weighted kappa value of 0.65 (CI 0.59-0.72) where extent of the lesion needed to be specified. Intra-observer agreement was also high, with weighted kappa values of 0.65 and 0.8 for presence of osteonecrotic lesions in the epiphysis. The presence of marrow oedema, punctate foci of altered signal, and mottled marrow changes were associated with a higher level of disagreement between observers. This study was conducted with two observers for validation of the proposed classification system, with review of only 36 imaging studies. Both observers were very familiar with ON of the knee, and their familiarity may have contributed to the high levels of agreement in categorising lesions.

The most recent categorisation system by developed by Niinimäki et al aimed to validate a MRI based radiological classification system which was suitable for any joint or bone, and in all patients with cancer [167] (Figure 6). In cases with multiple areas of ON, the grade is based on the most severe lesion. This classification system takes into account the mechanical properties of the bone, location of the lesion, and involvement of the articular surface. It was validated by assessment of MRI scans of 36 patients with ON (median age 27.5 years). Four independent observers reviewed the total of 72 MRI images. Inter-observer agreement for location of ON was determined to be very good, with kappa values from 0.93-0.98. Intra-observer agreement for classification of ON was good or very good, with kappa values from 0.79-0.86. Inter-observer agreement for classification of ON was lower, but still good, with kappa values from 0.62-0.77.

The validation of this classification system has a number of weaknesses. Only a total of 72 MRI scans from 36 patients were assessed, and all patients were likely to have been symptomatic, limiting assessment of asymptomatic lesions. This article also did not assess clinical prognostic value of the classification system. The median age of patient was 27.5

years, and whilst the youngest patient assessed was 11 years of age, the majority of patients were adult patients. Only 16 of the patients had ALL, and of those only 13 were under 24 years of age. However, this appears to be the system that fulfils the greatest number of criteria initially described for a clinically useful classification system in our patient population and further validation of this would be of value.

Figure 6. Niinimäki osteonecrosis classification system

Grade	Weight bearing bones:		Non-weight bearing bones:	
	Long bone	Short bone	Long bone	Short bone
0	No ON	No ON	No ON	No ON
1			Diaphysis or metaphysis (0%)	Body (0%)
2	Diaphysis or metaphysis (0%)	Body (0%)	Epiphysis (<30%)	Surface (<30%)
3	Epiphysis (<30%)	Surface (<30%)	Epiphysis (≥30%)	Surface (≥30%)
4	Epiphysis (≥30%)	Surface (≥30%)		
5	Deformation of joint	Deformation of joint	Deformation of joint	Deformation of joint

Area of articular involvement presented in brackets

The lack of a consensus definition for the classification of ON in ALL has been recognised internationally, and the Delphi method has been used by the Ponte di Legno working group (which consisted of 15 international childhood ALL study groups) to develop a consensus definition that aims to allow reliable comparisons of frequency and severity of ON across treatment protocols [168].

This classification system, presented in table 9, combines both radiological and clinical features of ON, and states that the disorder should be confirmed by MRI.

Table 9. Ponte di Legno consensus classification of osteonecrosis

Grade	Radiological and clinical features
1	Asymptomatic, with findings only by MRI.
2	Symptomatic, not limiting or only slightly limiting self-care activity of daily living. Lesions only outside joint lines in non-weight-bearing joints.
3	Symptomatic, not limiting or only slightly limiting self-care activity of daily living. Lesions in weight-bearing bones or affecting joint lines in non-weight-bearing bones.
4	Symptomatic with deformation by imaging of one or more joints and/or substantially limiting self-care activity of daily living.

2.1.5 The natural history of osteonecrosis in young patients with acute lymphoblastic leukaemia

When assessing and managing a patient with ON, it is crucial to understand the natural history of both symptomatic and asymptomatic osteonecrotic lesions, as this will influence how patients are counselled and managed.

2.1.5.1 Asymptomatic osteonecrosis

In the prospective study by the SJCRH team looking at ON in children with ALL, it was found that patients who were diagnosed with asymptomatic ON at initial screening were more likely to develop symptomatic ON (26%, compared with 14% of patients who were initially negative for ON, $p=0.008$) [132]. Of the 141 patients identified in the initial screen as having asymptomatic ON, follow up scans were available for 130 patients. Of these patients, 14 (11%) had lesions that resolved, 82 (63%) maintained their grade and 34 (26%) had worsening of their ON to grade 2 and 4 [132]. In the comparable study based in Egypt, of the 25 patients who were asymptomatic, (of whom 24 patients had grade 1 ON), follow up MRI scans were available for 16 patients. In these patients there was progression of ON in 4 patients (25%), resolution in 1 patient (6%), and a stationary course in 11 patients (69%) [129].

Another study by SJCRH retrospectively reviewed 109 patients with haematological malignancies who had routine MRI of knees, and confirmed ON [169]. In this study, reported by Karimova et al (2010), those who were asymptomatic at diagnosis of ON were less likely to have collapse and pain than those who were symptomatic (6% versus 37%) [169]. This study did not describe in detail the natural history of patients who were asymptomatic at first MRI.

The OPAL trial (Osteonecrosis in Pediatric patients with Acute lymphoblastic Leukemia and Lymphoblastic Lymphoma) is an ongoing German trial using prospective MRI screening at diagnosis and after 6 months [133]. Of 76 patients, 7 patients (9.2%) had asymptomatic ON at diagnosis, and at time of publication 5 had their follow up 6 month scan. All patients remained asymptomatic and in these 5 patients, the number of osteonecrotic lesions increased in one patient, decreased in 2 and remained constant in 2 others. At diagnosis, 5 of the 7 patients had grade 1 ON, and in 2 patients this increased to grade 2 (using the ARCO grading). One patient had grade 3 ON at diagnosis, which by 6 months had reduced to grade 1.

2.1.5.2 Symptomatic osteonecrosis

In a prospective study looking at long term outcomes of symptomatic ON, with a median follow up time of 4.9 years after diagnosis of ON, in 40% of cases symptoms resolved completely [141]. There were 24 patients who were available for analysis of radiological outcome, and in these patients 25% had partially or completely reversible lesions, 54% showed stable lesions, and 21% had progressive lesions. The study reported by Ali et al found that in the symptomatic group of patients, extensive (grade 4-6) ON developed in 14% of patients, and age >10 years was an independent risk factor for development of extensive ON [129].

In the study reported by Karimova et al (2010), 42 patients had symptomatic ON, with clinical information available for 36 patients. Joint collapse was experienced by 22% after the diagnosis of ON and the median time between the diagnosis of ON of the knee and collapse was 12 months, with a range of 2.1-97.1 months [169]. Of the 2 patients who had core decompression, one patient had collapse of the articular surface 3.5 years following surgery. In an Italian study of 99 cases of ON, 19.4% underwent arthroplasty, with 50% of patients undergoing one or more alternative interventions [122].

2.1.6 Risk factors for development and progression of osteonecrosis in young people with acute lymphoblastic leukaemia

2.1.6.1 Patient demographics

All studies found age at primary diagnosis to be a significant risk factor for the development of symptomatic ON. Patients aged <10 years at diagnosis were at much lower risk than older patients [109-112, 114-116, 119-122, 127, 128, 132, 145]. The study by Patel et al [123] found that adolescents younger than 20 years of age at diagnosis of ALL were at higher risk of ON compared with older patients. Taken together, these suggest that patients aged between 10 and 20 years at diagnosis of ALL are the group most prone to developing symptomatic ON, compared with any other age group. Age also appears to be a risk factor for developing more extensive ON lesions [129, 131]. In a study by the SJCRH, of patients identified with extensive lesions of the femoral head, 83% were older than 10 years (n=40), and of those, 48% (n=19) progressed to joint collapse requiring total hip arthroplasty [131]. A number of studies found the highest risk in the later teenage years (aged >15 years) [111, 134], but this was not a consistent finding throughout the studies. In the prospective MRI screening study

looking at extensive hip ON, patients aged 11-15 years had the same incidence of extensive ON as those older than 15 years of age[131], while in the NOPHO ALL 2008 study [142] where patients aged 1-45 years were treated, it was found that the highest risk group was patients 10-14 years of age. Older age at primary diagnosis was found to be associated with more frequent collapse, with one study finding 40% of adolescent patients with knee ON experienced collapse, compared with less than 4% of younger patients [169].

Despite age being almost universally accepted as a risk factor for development of ON, it should be noted that in the St Jude's prospective study, age was not found to be a risk factor for the development of asymptomatic ON [132]. This could suggest that all patients have the same risk of developing early ON lesions, but only older patients are at risk of these progressing to form extensive symptomatic lesions.

It has been suggested that female sex is a risk factor for the development of ON, but the literature around this topic is far from conclusive. A number of studies found female sex to be a risk factor [100, 109, 117, 119, 120, 134-136, 141, 144], whilst many others found it not to be [110, 111, 114, 123, 125, 127, 131, 140, 170], even when similar treatment regimens were used [109, 111]. One study found that female sex was a risk factor for development of ON only in the 10-18 year age group[120]. Even in groups with the highest rates of ON there are disparate results- the CCG study reported the disorder more frequently in females [119], whilst no gender differences were found in the DFCL ALL consortium [127] and studies at SJCRH [140]. In the study by Mattano in 2000 [119] the gender difference was greatest in the 10-15 year age group, with 3 year rates of 19.2% for females and 9.8% for males. However, among the smaller group of 16-20 year olds with ON, the incidence of ON was higher in males than in females (20.7% v 13.2% respectively). This could indicate the importance of pubertal stage, as males will typically have later onset and completion of puberty than females. It may also be that the specific treatment regimen influences the importance of sex as a risk factor in development of ON.

BMI was found to be a risk factor in only one study [136] where patients with a BMI >95th percentile at the end of treatment were found to have a higher risk of ON, but BMI at diagnosis was not found to be a risk factor in a number of other studies [110, 120, 131, 140, 141]. It is possible that the thresholds used for statistical analysis could influence whether or not BMI was found to be a risk factor for development of ON.

White race was found to be a risk factor in a number of studies [117, 119, 146], but this was not a consistent finding [131, 132]. Ethnicity as a risk factor is a difficult area to study due multiple confounding factors, variation in terminology and differences in how ethnic groups are categorised. A number of studies separated patients only into White and non-White, whilst others had Black and Hispanic groups. Few studies commented on Asian patients and most studies where race was commented upon were composed of predominantly White patients.

2.1.6.2 Treatment for acute lymphoblastic leukaemia

Glucocorticoids are recognised as a causative factor in the development of ON in children and young people with ALL. Therefore, it is logical to consider whether dose, type and timing of corticosteroid in ALL treatment affects the prevalence of ON.

Not all studies described the cumulative corticosteroid dose received by patient, and there are often differences depending on treatment arm. Additionally, steroids may be used outside of the ALL treatment protocol: in some centres dexamethasone was used as a treatment for nausea and vomiting or as prophylaxis during cranial radiation therapy [136]. There were also considerable variations in time schedule of steroid administration. In some regimens steroid administration was restricted to induction and intensification, whilst other regimens have steroids administered during maintenance therapy, making the duration of steroid exposure considerably longer. A number of studies did describe the treatment regimen and cumulative corticosteroid dose in detail. In two studies with similar age distribution and patient population, the study with the higher corticosteroid dose found a significantly higher prevalence of ON [127] compared with the study where corticosteroid doses could be up to 3 times lower [111]. However, these were both retrospective studies, with different methodologies used for confirming diagnosis of ON. In the prospective study by Niinimaki it was found that patients with ON had received significantly more dexamethasone compared with those without ON [136], but the odds ratio was only 1.01, with confidence intervals (CI) of 1.00-1.01, and the study by Ribeiro et al [140] found no difference in cumulative dose of corticosteroids and risk of ON.

Type of steroid used also varies between protocols and could have an impact on the incidence of ON. It has been suggested that dexamethasone increases the likelihood of developing ON compared with prednisolone [114], but this is not a consistent finding [100, 110, 127]. A systematic review and

meta-analysis looked at the use of dexamethasone versus prednisolone for induction therapy in childhood ALL [171] and found that whilst there was clear evidence that dexamethasone was protective against CNS relapse, the incidence of ON did not significantly differ between the two corticosteroids. There were some limitations to this meta-analysis, namely that inclusion of older studies could result in underreporting, as accurate ascertainment of ON was limited, and there was no subgroup analysis to assess the role of age at primary diagnosis in influencing the impact of steroid formulation on incidence of osteonecrosis. It was also not possible to remove confounders, such as chemotherapy intensity and differences in patient population. US studies have found that the risk of ON increased in patients treated with dexamethasone compared with prednisolone [105]. Of UK studies, only the study looking at skeletal morbidity in patients treated in trials UKALL97, UKALL97/01 and UKALL2003 [114], found an increased incidence of ON when patients were treated with dexamethasone compared with use of prednisolone. The much larger prospective study looking at results from UKALL97 and UKALL97/99 [100] found no excess of ON in the dexamethasone arm of the trial.

A number of trials found ALL risk group to be a risk factor for development of ON, with more patients in high risk or moderate risk arms developing ON than those in low risk arms [109, 111, 118, 132], although a number of smaller studies found no significant difference between treatment arms [110, 127, 146]. Initial risk stratification can vary between treatment regimens, but as described previously, the current UK ALL study, UKALL 2011, which uses the NCI risk stratification, high risk at diagnosis is defined as patients ≥ 10 years of age and/or white cell count of $\geq 50 \times 10^9/L$, and all T-cell ALL and lymphoblastic lymphoma patients. In a large prospective study patients in the standard/high risk treatment arm were at higher risk of developing symptomatic ON compared with low risk patients (odds ratio 2.5), although there were wide CIs of 1.2-4.9 [132]. Patients in the standard/ high risk group received more intensive therapy, with dexamethasone at $12\text{mg}/\text{m}^2/\text{day}$ and 20 weeks of continuous ASP, compared with $8\text{mg}/\text{m}^2/\text{day}$ of dexamethasone and only 6 weeks of ASP in the low risk arm.

A number of studies noted that certain components of the treatment regime other than steroids also increased the risk of development of ON. It is possible that ASP may increase risk of development of ON due to a number of different mechanisms, including increasing lipid abnormalities [172], increasing thrombotic risk [173], and increasing plasma exposure to

dexamethasone. [132, 174]. In a study by Karol et al [117] patients with ASP allergy (and hence reduced exposure to ASP) were less likely to develop ON, and it is possible that ASP treatment potentiates steroid induced necrosis [174]. Hypoalbuminaemia is a marker of ASP treatment, which could explain why low serum albumin levels at week 7 were found to be a risk factor for development of ON in the study by SJCRH [132]. However, murine models have found that discontinuous dexamethasone has synergistic anti-leukaemic activity with ASP, without increasing incidence of ON [175]. One study found the use of pegylated ASP, versus E.coli or Erwinia ASP, was a significant risk factor for development of ON [110], but this was a small study and the significance of this finding is unclear. A study of 625 patients compared the use intermittent versus continuous pegylated ASP, and found no significant difference in hazard rate or cumulative incidence of symptomatic ON [176]. However, patients in this study had a median age of 4.2 years, with only 29 patients in total affected by ON. There was a trend towards a reduction in ON in the experimental (intermittent) arm, hence statistical significance may have been achieved in a larger study.

The specific treatment regimen used is an important factor in development of ON. The CCG1961 trial evaluated components of therapeutic intensification in high-risk patients (white cell count $\geq 50 \times 10^9$ and/or age ≥ 10 years) [106, 134]. It was found that use of alternate week rather than continuous dexamethasone during DI in high risk ALL patients results in a 2-fold reduction in the relative risk of symptomatic ON among rapid responders aged ≥ 10 years at diagnosis of ALL. There was a four-fold reduction among those randomised to intensified therapy, despite those with alternate week dexamethasone having a higher total dexamethasone exposure. The incidence of ON was lower among slow responders age ≥ 10 years assigned to double DI with alternate-week dexamethasone when compared with a similar cohort on the CCG1882 trial [119] who were assigned to two DI phases with continuous dexamethasone (11.8% versus 23.2%). The results of this study could indicate that dosing manner supersedes cumulative exposure.

2.1.6.3 Radiological risk factors

After diagnosis of ON in a patient with ALL, it is important to understand what the likely prognosis is for a specific lesion, in order to provide information for family and clinicians.

A number of classification systems emphasised the clinical importance of osteonecrotic involvement of the articular surface [159, 165-167]. Studies

have found involvement of the articular surface is associated with risk of progression [118], and femoral epiphyseal lesions extending to the articular surface were associated with a higher frequency of collapse [169].

Size of lesion has also been shown to influence the clinical course of the lesion, with larger size associated with risk of progression [118]. One team looked at the natural history of ON of the femoral head in 80 patients with a primary diagnosis of haematological malignancy [177]. In multivariable analysis it was found that the outcome of ON was solely predicted by lesion size at diagnosis, with worst prognosis associated with lesions occupying more than 30% of the femoral head volume. Of this group of patients, 80% of hips collapsed within 2 years of diagnosis, and 50% required arthroplasty.

A study assessing radiological features of ON in paediatric and adolescent (age <21 years) leukaemia patients, found that bone marrow oedema was a sign of progressive ON and eventual bone collapse [178]. In this retrospective study MRI images of 15 patients with epiphyseal ON in weight bearing joints were assessed, with 47 lesions evaluated. There were 17 cases of eventual bone collapse, and presence of bone marrow oedema had a sensitivity and specificity of 94% and 77% respectively, with significant association between presence of oedema on imaging and eventual disease progression. However, it should be noted that bone marrow oedema was only found in patients with lesions extending over 30% of the articular surface, which as discussed, is itself likely to be a poor prognostic factor, and 70% of patients with bone marrow oedema also had a subchondral fracture.

2.1.6.4 Genetic risk factors

A number of studies looked at genetic risk factors influencing development of ON. Earlier studies used a candidate gene approach to determine genotypes for common polymorphisms in genes likely to affect development of ON in a patient undergoing ALL treatment [144, 146]. A disadvantage of this approach is that suitable candidate genes are selected using existing knowledge about known or theoretical mechanisms of development of ON, and results are dependent on the selection of appropriate genes. The approach has been shown to produce a high rate of false positives [179], with results often failing to be replicated in follow up studies.

One study using the candidate gene approach reported by Relling et al found that low thymidylate synthase activity 2/2 (TYMS 2/2) enhancer repeat genotype and the vitamin D receptor Fok1 (VDR Fok1) start site CC

genotype were independent risk factors for ON of the hip in children undergoing treatment for ALL [146]. It found that the four risk factors of age over 10 years, White race, the TYMS 2/2 genotype and the VDR Fok1 CC genotype together have a sensitivity for predicting the development of ON of 96%, and a specificity of 82%. These gene products affect the pharmacodynamics, rather than the pharmacokinetics, of anti-leukaemic medications. Methotrexate inhibits thymidylate synthase activity by interfering with the ability of the enzyme dihydrofolate reductase to replenish intracellular stores of reduced folates, which is required by thymidylate synthase to act, and methotrexate polyglutamates also directly inhibit thymidylate synthase [180]. Cells that are TYMS 2/2 have increased sensitivity to methotrexate and patients with this allele may be more susceptible to methotrexate induced toxicity. The relationship between thymidylate synthase activity and ON was validated in a separate larger study (n=615) [181], but the association with VDR Fok1 genotype was not replicated. In this study TYMS 2/2 genotype was only associated with an increased risk of ON in patients younger than 10 years of age, with 10.7% of those with this genotype developing ON, compared with 4.1% those without. This may be because of the different treatment protocols used for those under and over 10 years of age, with patients under 10 exposed to more methotrexate in the first year of treatment [181]. In a different study looking at 12 candidate polymorphisms TYMS 2/2 and VDR Fok1 were not found to be risk factors for the development of ON [144]. These differences may again be due to differences in study protocols, with patients in the study by Relling et al receiving more anti-metabolites than those in other studies [144, 146]. These results could suggest that genetic risk factors depend upon the specific treatment therapy used.

Later genome wide association studies (GWAS) were used to identify genetic variants associated with development of ON in young people with ALL. GWAS studies have the advantage that they investigate the entire genome, but whilst they can identify single-nucleotide polymorphisms (SNPs) and other variants in DNA associated with a disease, they cannot specify causal genes.

In the largest GWAS of glucocorticoid-induced ON in children with ALL a number of genetic variants were identified as risk factors for development of ON [117]. Meta-analysis was performed by combining GWAS results from both the discovery cohort and the two validation cohorts. 197 SNPs with *P* values < 0.0001 were annotated to 64 genes. It was found that the

glutamate receptor signalling pathway, including three genes GRIN3A, GRIK1 and GRM7, was the top canonical pathway ($P = 4.8 \times 10^{-4}$), suggesting its involvement in the pathogenesis of ON in childhood ALL. It is possible that glutamate and variations in glutamate receptors may contribute to a proximal vascular event that increases the risk of ON in patients exposed to steroids, as genetic variation in GRIN3A has previously been associated with the severity of vascular complications in Kawasaki disease [182].

The same team looked at genetic risk factors for the development of ON in children under the age of 10 years treated for ALL [145]. The genes for BMP (bone morphogenic protein) 7 and PROX1-AS1 variants met the genome-wide significance threshold of $<5 \times 10^{-8}$. BMP7 is released in response to bone damage in osteoarthritis and spondyloarthritis, and its release is increased by mechanical stress [183, 184]. BMPs can induce mesenchymal precursor cells to differentiate into osteoblasts and inhibit the formation of osteoclasts [185]. Variants affecting this gene could contribute to development of ON by altering bone metabolism and formation both prior to and during ALL therapy. BMP7 is also known to be toxic to vascular smooth muscle [186], and therefore could contribute to local arteriopathy resulting in ischaemia, and hence ON. BMP7 expression in the absence of vitamin D induces osteoblast differentiation and mineralization, but this is reversed in the presence of 1,25(OH) vitamin D [187]. BMP7 was not identified in previous genome wide studies looking at associations with ON. This may reflect differences in the populations studied, with BMP7 a specific ON risk in younger patients.

PROX1 has been shown to control the differentiation of lymphatic endothelial cells from vascular endothelial cells [188], and has also been noted to be down-regulated in familial combined hyperlipidaemia [189]. This could result in reduced clearance of plasma lipids, which would increase the risk of development of ON.

The top 92 validated SNPs were enriched for locations within enhancers active in tissues closely related to ON, specifically mesenchymal progenitors [145]. These cells can differentiate into either osteoblasts or adipocytes. SNPs significantly associated with ON were linked to 7 genes in the adipogenesis pathway, supporting the importance of genes affecting mesenchymal differentiation in ON.

In pathway analysis, there were 3,271 SNPs with significant ($p < 0.05$) associations with ON [145]. The 459 genes to which these SNPs annotated

were significantly enriched within 8 canonical pathways. Of these, 7 contained glutamate receptor genes, with the glutamate receptor signaling pathway most overrepresented. Variants in 6 genes in the glutamate receptor pathway were associated with the development of ON in this cohort, including the top validated non-synonymous variant (rs34144324 in GRID2). This signaling pathway was also the top pathway represented by genetic variants in a cohort of high risk patients of all ages. The adipogenesis pathway was the only overrepresented pathway whose genes did not overlap with glutamate receptor signaling.

An alternative method of assessing genomic variation in development of ON is the Projection Onto the Most Interesting Statistical Evidence (PROMISE) integrative analysis technique, which uses data defining the biological inter-relationships of phenotypes with one another. This was used to determine genetic variants associated with pleiotropic dexamethasone phenotypes (where genetic variation at a single locus has an effect on more than one phenotype). The focus was on ON and thrombosis, and results were compared with single phenotype GWAS [190]. This identified more risk variants for glucocorticoid effects in regulatory regions than single phenotype analysis, and 5 of the top 10 SNPs were chromosome 12 near keratin genes, with 4 of these 5 in linkage disequilibrium with SNPs in a glucocorticoid receptor-binding site. When the SNPs were prioritized the top scoring SNP was one downstream of F2RL1 (rs6453253), which was near another selected SNP in the intron of F2RL1(rs2243057). Both of these SNPs were in the regulatory region in osteoblast cell lines, and rs6453253 was also in a glucocorticoid receptor binding site. The G allele of this SNP was associated with an increased risk of osteonecrosis, increase in cholesterol and higher dexamethasone exposure. The A allele of rs2243057 was associated with an increased risk of ON and thrombosis, with lower albumin level and a greater increase in cholesterol and triglycerides from week 7 to week 8 of continuation therapy. The advantage of this method of analysis is that by accounting for pleiotropic effects the probability of selecting variants that exert their effect through common mechanisms (e.g. glucocorticoid responsive transcriptional machinery) is enhanced.

In the prospective study by the SJCRH team [132] SNP genotyping was performed. 423 SNPs were associated with symptomatic ON, and of these the top 4 SNPs were in the SH3YL1-ACP1 gene locus. Of the 423 SNPs, 27 were associated with low albumin or high cholesterol. ACP1 is associated with serum cholesterol and triglyceride levels [191], and regulates osteoblast

differentiation [192]. Higher serum cholesterol and lower serum albumin were associated with grade 2-4 ON. This study suggested that ACP1 may act via multiple mechanisms to affect bone homeostasis. A study by French et al [144] found a polymorphism in SERPINE 1 to be associated with development of ON, but this was not found to be the case in the more recent study [132], and other studies have found ON to be associated with SNPs located within the BCL211 gene, which may affect osteoblast and osteocyte apoptosis [147, 193]

2.1.6.5 Additional findings

There are a number of additional findings in singular studies.

In the patients recruited into the prospective study conducted by SJCRH a significant association between development of bacteraemia and subsequent development of symptomatic ON ($p=0.038$, CI 1.03-3.41) was found [194]. Although this association remained significant after adjustment for race and gender, it did not remain so after adjustment for age, which is one of the most significant risk factors for development of ON. Nonetheless, there was found to be an association between increased number of episodes of bacteraemia and development of symptomatic ON, which remained after adjustment for race, gender and age ($p=0.04$) [194]. This finding has not been assessed in other studies, and hence it is difficult to draw any firm conclusions. However, that episodes of bacteraemia could compromise the integrity of the vascular supply to vulnerable areas of bone is biologically plausible.

One study looked at the association between development of ON and changes in BMD during treatment for ALL [130]. It was found that lumbar spine and total body BMD were not different at baseline between patients who did or did not develop ON, but at cessation of treatment and one year after cessation of treatment, patients with ON had significantly lower mean BMD than patients without. Patients with ON were also more likely to have BMD <-1 SDS and -2 SDS at cessation of treatment (lumbar spine BMD (LSBMD) SDS <-1 in 90% of patients with ON, versus 60% of patients without ON, and <-2 in 62% of patients with ON, versus 25% of patients without ON). This reduction in BMD may be due to a combination of avoidance of weight bearing activities and the ON itself. This suggests that patients who develop ON during treatment for ALL may be in need of extra medical care for management or prevention of low BMD.

An important negative finding in a large prospective study looking at both asymptomatic and symptomatic ON of the hip is that risk of ON was not associated with patency of the epiphysis [131]. One might expect that the association of ON with age is due to lack of physeal patency of older patients, but this study did not suggest this to be the case.

As development of ON is due to an interruption of the vascular supply to an area of bone, it is plausible that patients with increased likelihood of clot development would be at increased risk of development of ON. A conference abstract described how induction therapy related alterations in coagulation may be associated with development of ON [56], with levels of antithrombin (AT) and protein S significantly less in ON positive than in ON negative patients after 4 weeks of treatment, including dexamethasone. (161 paediatric patients). The study by Badhiwala et al [110] found that thromboembolism during treatment was a significant predictor of ON, and supports a role for hypercoagulability in the pathogenesis of ON. However these results are difficult to interpret due to lack of detail provided in the reports described.

Hypertension in patients with ALL may also be postulated to increase likelihood of development of ON, due to potential increased pressure within the bone marrow. A recent conference abstract presented at société internationale d'oncologie pédiatrique (SIOP) 2018 found that in an assessment of 60 patients over the age of 10 years, hypertensive children were at greater risk of developing ON [195]. A murine model found that use of quinapril to reduce blood pressure reduced the development of ON [195].

2.1.7 Summary

Osteonecrosis is increasingly recognised as a common cause of morbidity in patients being treated for ALL. Prospective studies suggest that asymptomatic ON affects up to 54% of patients, whilst symptomatic ON affects around 18% of patients [132]. Most patients who develop symptomatic ON do so within the first 3 years after diagnosis, but asymptomatic lesions are likely to develop earlier, and studies suggest they develop within the first year of treatment [131, 132].

A significant proportion of young people affected by ON will have lesions that will spontaneously resolve. Patients who have asymptomatic ON early in treatment appear to be more likely to go on to develop symptomatic ON, but although some risk factors are well elucidated, the specific reasons for progression or regression of lesions is not clear. It is recognised that

symptomatic and progressive ON is more likely if there is involvement of the articular surface and a larger osteonecrotic lesion, and the presence of bone marrow oedema may also increase likelihood of progression of ON.

It is clear that older age at diagnosis of ALL is an important risk factor for the development and extension of symptomatic ON lesions, but studies have shown inconsistencies in other risk factors. It is not clear if incidence of ON increases with dexamethasone or prednisolone, and other chemotherapeutic agents such as ASP are also likely to have a role in development of ON. The manner of steroid dosing has been shown to affect likelihood of development of ON, with alternate week rather than continuous dexamethasone during DI in high risk ALL patients resulting a reduction in risk of development of ON.

A number of genetic factors have been highlighted as increasing the risk of development of ON, and genes affecting the glutamate receptor pathway, osteoblast regulation and adipogenesis may be of particular importance.

A crucial difficulty in discussions about radiological features of ON is that whilst multiple grading systems for ON exist, the majority have limitations when used in the context of paediatric patients with ALL. The classification system devised by Niinimaki et al [167], which was developed specifically for patients with cancer, and is applicable to all areas of ON, may have clinical relevance in our patient population but needs further validation, particularly with respect to prognostic significance.

2.2 Bone mineral density changes in children and young people with acute lymphoblastic leukaemia

Bone fragility is an important aspect of bone health, and this section reviews the medical literature around BMD and fracture risk of young people with ALL.

2.2.1 Research questions

Within this review I aim to cover the following questions:

1. What is the BMD of ALL patients at diagnosis and during treatment of ALL, when compared with healthy peers?
2. What is the BMD of patients treated for ALL after completion of therapy, when compared with healthy peers?
3. What are the risk factors associated with low BMD in patients with ALL?
4. What is the fracture risk of patients during and after treatment for ALL?
5. What are the risk factors associated with increased fracture rate in patients with ALL?
6. What is the natural history of vertebral fractures in patients with ALL?

2.2.2 Search Strategies

Medline 1996-2017, Embase 1996-2017, EBM databses, Journals @Ovid, Books@Ovid were searched. In addition I searched the reference lists of relevant studies.

Keywords were osteoporosis OR fractures OR bone mineral density, which was combined with AND 'leukaemia' as a MeSH term.

Duplicate references were manually removed and eligibility judgements were made on the basis of information found in the article abstract and full article when appropriate.

My initial search had a yield of 501 articles. Of these 26 were found to be relevant to my study question. During the research period weekly reports from Ovid and Embase with any of the above terms in the abstract, title or as a keyword were reviewed and added, and the literature review was last updated in May 2019.

Studies which were presented only as abstracts were included only if sufficient information was available in the abstract, or after contact with the author.

The tables below summarise the most pertinent studies. Table 10 presents data from studies in which patients had an assessment of BMD during ALL treatment, Table 11 presents data from studies in which patients were assessed after completion of ALL therapy, and Table 12 summarises the key studies assessing fracture risk in patients with ALL.

Table 10. Studies assessing bone mineral density of patients during treatment for acute lymphoblastic leukaemia

First author, Country Year of publication	Patient inclusion criteria	Number of patients	Protocol (recruiting period)	Study design, timing, BMD assessment (method)	Bone mineral density
Alos et al. Canada 2012 [196]	Newly diagnosed ALL aged 1 month-17 years	188	Treatment protocol: 9 sites COG, 1 site DFCI protocol (2005-2007)	Prospective screening of asymptomatic patients within 30 days of ALL diagnosis and at 12 months LSBMD (DXA)	Baseline LS-BMD Z score -1.2 (SD 1.3), change at 12 months 0.1 (SD 0.9)
Cummings Canada 2015 [41]	Newly diagnosed ALL aged 1 month-17 years	188	Treatment protocol: 9 sites COG, 1 site DFCI protocol (2005-2007)	Prospective screening of asymptomatic patients at diagnosis then annually for 4 years LSBMD (DXA)	Baseline LSBMD Z score -1.2 +/-1.3, (P<0.001 compared to healthy average), increased to -1.1 at 1 year, -0.7 at 4 years
de Hoed Holland 2015 [130]	Newly diagnosed ALL aged 4-18 years	466	DCOG-ALL9 (1997-2004)	Prospective screening of asymptomatic patients at diagnosis, 32 weeks, end of treatment and 1 year after cessation of therapy LSBMD (DXA)	At cessation of treatment mean LSBMD -1.28 SDS; no values given for other LSBMD time-points
Halton Canada 1996 [197]	Newly diagnosed ALL aged 0-17 years	40	DFCI 87-01 (not documented)	Prospective screening of asymptomatic patients at diagnosis and at 6 monthly intervals for 2 years LS BMD (DXA)	Compared with status at diagnosis, Z scores for BMD and BMC were not statistically significantly different throughout therapy 47% of patients had reduction in BMD from baseline
Inaba USA 2018 [198]	New diagnosis of ALL aged 1-18 years	363	Total XV therapy (2000-2007)	Prospective screening of asymptomatic patients at diagnosis, completion of therapy and 2 years after completion of therapy LSBMD (QCT)	Diagnosis: median BMD Z score 0.06 End of therapy median BMD z score -1.08 2 years after therapy: BMD z score -0.72 No indication of statistical difference between patients with ALL and reference group

First author, Country Year of publication	Patient inclusion criteria	Number of patients	Protocol (recruiting period)	Study design, timing, BMD assessment (method)	Bone mineral density
Kohler UK 2012 [199]	Undergoing ALL treatment, age > 4 years	39	UKALL 2003 (not specified)	Cross-sectional study of patients compared to healthy controls. BMD assessed at 100±45 weeks since diagnosis Femoral neck and LSBMAD (DXA) Radial and tibial volumetric BMD (pQCT)	48.6% of ALL patients had femoral BMAD <-2.0 SDS, 18.4% had LS-BMAD <-2 SDS. BMAD significantly lower in ALL patients than controls Radial and tibial trabecular vBMD reduced compared to controls (p=0.03) but cortical vBMD at radius and tibia similar in patients and controls
Ness USA 2015 [200]	Newly diagnosed ALL aged 4-18 years	109	Not specified (2009-2013)	BMD assessment of asymptomatic patients at diagnosis of ALL LSBMD (DXA)	LS-BMD mean SD -0.45 at diagnosis p=0.01 when compared to control
Orgel USA 2016[201]	Newly diagnosed ALL, aged 10-21 years	38	COG protocols AALL0232, AALL1131, AALL0434 (not specified)	BMD assessment of asymptomatic patients at diagnosis and end of induction LSBMD, femurs and tibias BMD (QCT) Whole body BMD (DXA)	No difference at diagnosis in aBMD (TBLH) between patients and controls (z-score 0.28). aBMD TBLH after induction decreased by -2.5%(CI - 3.2—1.8)
Rayar Canada 2012[202]	ALL in consolidation Aged ≤18 years	124 (46 patients had diagnosis DXA)	DFCI 91-001, 95-001, 20-001, 05-001 (1995-2006)	BMD assessment of asymptomatic patients at diagnosis and during consolidation phase of therapy Lumbar spine BMD (DXA)	Median change in LS BMD Z score -0.08
te Winkel Holland 2014 [203]	Newly diagnosed ALL. age over 4 years	399 (BMD study) 672 (fracture study)	DCOG-ALL9 protocol (1997-2006)	BMD assessment of asymptomatic patients at diagnosis, after 32 weeks, after 2 years (at cessation of therapy) and 3 years. LSBMD (DXA)	At diagnosis: mean LSBMD -1.1 SDS 8 months: LSBMD -1.1 24 months: LSBMD -1.27 36 months: LSBMD -0.95

Table 11. Studies assessing bone mineral density of patients after completion of acute lymphoblastic leukaemia therapy

Author Country Year of publication	Patient inclusion criteria	Number of patients	Protocol (treatment/ recruiting period)	Study design, timing, BMD assessment (method)	Bone mineral density (comparison with healthy population)
Arikoski Finland 1998 [204]	Survivors of childhood ALL	29	NOPHO protocols between 1972-1991 (not documented)	Cross sectional; BMD assessment median 8 years after cessation of therapy (range 2-20 years); LSBMD and femoral neck BMD (DXA)	No significant difference in BMD compared with healthy controls
Arikoski Finland 1999 [205]	Survivors of ALL; age 1-16 years	22	NOPHO protocols (1995-1997)	Cross sectional; BMD assessed at cessation of chemotherapy; LSBD and femoral neck BMD (DXA)	Significant reduction in BMD compared with healthy controls LS volumetric BMD mean Z score -0.77, CI - 1.3—0.23, femoral volumetric mean Z score - 0.98, CI -1.6- -0.32.
Benmiloud Belgium 2010 [206]	Survivors of childhood ALL and NHL; >5 years after remission; age 16 -32 years	89 (74 patients with ALL)	FRALLE protocols, ALCL protocol for NHL (not documented)	Cross sectional; BMD assessed mean 15±4.5 years after cessation of chemotherapy; LSBMD, femoral neck, hip BMD (DXA)	40% of patients had LSBMD ≤-1 (10% ≤-2). Low BMD at femoral neck in 24% of patients.
Brennan UK 1999 [207]	Male survivors of childhood ALL	31	UKALL I-X or Memphis V. All patient had cranial irradiation (not documented)	Cross sectional; BMD assessed median 17.8 years after cranial irradiation (6.8- 28.6 yrs); LSBMD (QCT); LSBMD and right femoral neck (DXA)	Reduced BMD compared with healthy peers. QCT: median Z-score -1.25 (range -3.51-0.95, p<0.001), DXA: LS-BMD median Z score -0.74, range -2.1-1, p=0.001
Brennan UK 2005 [208]	Survivors of childhood ALL	53	UKALLXI (not documented)	Cross sectional; BMD assessed median 4.6 years after cessation of treatment (range 1.2-8.3 years); LSBMD and TBBMD (DXA) Distal and mid-radial sites (pQCT)	No difference in total body or LS BMAD compared to controls.
den Hoed Holland 2014 [209]	Survivors of childhood cancer> 5 years after end of chemotherapy	142 patients with ALL (346 patients in total)	Varying protocols, not specified (Patients treated between 1965-2003, enrolled 2003-2008)	Cross sectional; BMD assessed median 16.7 years after cessation of chemotherapy; LS and whole body (DXA)	Lower LSBMD and TBBMD compared to healthy peers. Mean LS BMD -0.3 SDS, mean total body SDS - 0.55

Author Country Year of publication	Patient inclusion criteria	Number of patients	Protocol (treatment/ recruiting period)	Study design, timing, BMD assessment (method)	Bone mineral density (comparison with healthy population)
Gurney USA 2014 [210]	Survivors of childhood ALL \geq 10 years post diagnosis; age $>$ 18 years	845	Varying protocols, not specified. (Sept 2007-Oct 2012)	Cross sectional; BMD assessment median 26 years after cessation of chemotherapy; LSBMD (QCT)	BMD Z score \leq -2: 5.7%, Z score -1 to -2: 23.8%
Jain India 2016 [211]	Survivors of childhood ALL; $<$ 18 years at diagnosis of ALL; $>$ 2 years from completion of chemotherapy	65	ALL therapy MCP-841 or BFM-95 (treated between 1996 and 2008)	Cross-sectional; BMD assessment median 4.3 years (range 2-14.8 years) after cessation of chemotherapy; LSBMD and TBBMD (DXA)	No significant difference in height adjusted lumbar or whole body BMD between patients and age and sex matched controls
Le Meignen France 2011 [212]	Survivors of childhood ALL or AML	159 (130 patients treated for ALL, 29 for AML)	Various non-specified French multicentric protocols. Treated from 1980-2011 (2007-2008)	Cross sectional; BMD assessment mean 14.66 \pm 0.44 years after diagnosis of malignancy; LSBMD, femoral neck BMD (DXA)	Mean femoral neck Z score -0.19 \pm 0.08. Lumbar spine BMD mean Z score -0.37 \pm 0.08
Makitie Finland 2013[213]	Male survivors of childhood ALL ($>$ 10 years post diagnosis)	49	Protocol not specified. ALL treatment between 1970-1998, (not specified)	Cross sectional; BMD assessment 10-38 years after ALL diagnosis; LS, total body, hip, femoral neck BMD (DXA)	Reduced whole body BMD Z score in ALL survivors. No significant difference in lumbar spine BMAD, femoral neck or total hip BMD Z-score
Mostoufi-Moab USA 2018 [40]	Survivors of childhood ALL, within 2 years of completing therapy. Age 5-18 years	45	Children's Oncology Group Consortium	Prospective longitudinal study; BMD assessment median 0.8 years after cessation of chemotherapy and 12 months later; LSBMD, TBBMD, hip, forearm BMD (DXA) Tibia (pQCT)	No difference in TBLH/ LS BMD at baseline compared with reference data. Significant increase in BMD after 12 months
Rai USA 2008 [214]	Survivors of childhood ALL, $>$ 5 years from completion of therapy	424 in baseline study	Total Therapy XI, XII and XIII. (treated between 1984-1997)	Cross sectional study; Average age of BMD assessment not documented. LSBMD (DXA and QCT)	Median age- and gender-specific LS-BMD Z-score was -0.3 (-3.7 to 3.2) for females and -0.6 (-3.9 to 5.1) for males

Table 12. Studies assessing fractures in patients with acute lymphoblastic leukaemia

First author, Country Year of publication	Patient inclusion criteria	n	Protocol (recruiting period)	Study design and method of fracture measurement	Fracture prevalence
Alos Canada 2012 [196]	Newly diagnosed ALL aged 1 month-17 years	155	9 sites COG, 1 site DFCI (2005-2007)	Prospective screening of asymptomatic patients at diagnosis and 1 year. VF (lateral spine radiographs)	16% 1 year after diagnosis of ALL
Cummings Canada 2015 [41]	Newly diagnosed ALL aged 1 month-17 years	188	9 sites COG, 1 site DFCI (2005-2007)	Prospective screening of asymptomatic patients at diagnosis then annually for 4 years VF (lateral spine radiographs)	4 year cumulative incidence 26.4%
Halton Canada 1996 [197]	Newly diagnosed ALL, aged 0-17 years	40	DFCI 87-01 (not documented)	Prospective screening of asymptomatic patients at diagnosis and at 6 monthly intervals for 2 years VF (lateral spine radiographs)	39%
Halton Canada 2009 [215]	Newly diagnosed ALL aged 1 month-17 years	186	9 sites COG, 1 site DFCI (2005-2007)	Prospective screening of asymptomatic patients within 30 days of ALL diagnosis. VF (lateral spine radiographs)	16% at diagnosis
Rayar Canada 2012 [202]	ALL in consolidation Aged ≤18 years	124	DFCI 91-001, 95-001, 20-001, 05-001 (1995-2006)	Assessment of symptomatic fractures during chemotherapy. No description of method of fracture assessment.	18.5%
te Winkel Holland 2014 [203]	Newly diagnosed ALL, age > 4 years	672	DCOG-ALL9 protocol (1997-2006)	Assessment of symptomatic fractures from diagnosis to one year after cessation of treatment. Radiological assessment of symptomatic areas.	17.8% cumulative incidence at 3 years
Ward Canada 2018 [216]	Newly diagnosed ALL aged 1 month-17 years	186	9 sites COG, 1 site DFCI (2005-2007)	Prospective screening for asymptomatic VFs at baseline then annually for 6 years. VFs (lateral spine radiographs) Symptomatic low trauma non VFs confirmed by radiology	VF: 32.5% cumulative incidence Non VF: 23% cumulative incidence

2.2.3 Bone mineral density at diagnosis and during treatment of acute lymphoblastic leukaemia

There are 3 main studies which address the question of bone mineral density of patients with ALL at diagnosis and during treatment, compared with the healthy population. The largest is a Dutch study, with results presented by den Hoed et al [130] and te Winkel et al [203]. In this study LSBMD was assessed by DXA in 399 patients with ALL. BMD was measured at diagnosis, after 8 months, after 2 years (which was after cessation of therapy) and after 3 years. At diagnosis, BMD was significantly lower than that of healthy peers, with a mean lumbar spine SDS of -1.1 ($p < 0.001$). At 8 months, LSBMD remained at -1.1 SDS ($p < 0.001$). By 24 months this reduced to -1.27 SDS ($p < 0.001$) but recovered slightly by 36 months (BMD = -0.95 SDS), although it still remained significantly lower than the BMD of the healthy population ($p < 0.001$) [130, 203].

Consistent with this, a Canadian study, presented variously in papers by Alos et al [196], Cummings et al [41] and Halton et al [215], also found lower baseline BMD Z-scores at diagnosis of ALL when compared with healthy peers. This study, known as the Steroid Associated Osteoporosis in the Paediatric Population (STOPP) research program, assessed LSBMD by DXA within 30 days of diagnosis and every 6 months for 4 years. There were 186 patients recruited at baseline, which reduced to 136 by 4 years. Baseline Z-scores were lower than that of healthy peers, with a Z-score of -1.2 ± 1.3 ($p < 0.001$) [41]. The score increased after the baseline assessment, with a Z-score of -1.1 ± 1.1 at 1 year, increasing to -0.7 ± 1.2 at 4 years [41]. However, numerical data, with level of statistical significance compared with the healthy population, was not reported for each time-point [41].

The other main study, conducted by SJCRH, used QCT to assess lumbar spine vertebral trabecular BMD [198]. As previously discussed in Chapter 1.2.2, QCT assesses volumetric BMD, and is independent of bone size. Trabecular bone is the more metabolically active area of the bone, and hence is considered a more sensitive indicator of skeletal metabolism than cortical bone. Patients were assessed at diagnosis, week 120 of continuation therapy (end of treatment for females), week 146 of continuation therapy for male patients (end of treatment for males) and 2 years after completion of therapy. BMD data was available for 340 patients at diagnosis, and 232 patients by 2 years off therapy. The median BMD Z-score was 0.06 (range -3.27 to 3.56) at diagnosis, which decreased to -1.08 (range -5.93 to 2.05) by week 120 but improved to -0.72 (range -3.56 to

2.46) at 2 years after therapy. This was the only large study where BMD at diagnosis was not lower than healthy controls, with patients ≥ 10 years of age found to have a median BMD Z-score of 0.5, which was higher than normal controls, whilst those aged 2-9.9 years had a median Z-score of -0.09, which was comparable with controls [198]. This may be a reflection of the method of BMD measurement, or of the specific population assessed.

Other smaller studies using DXA assessment also found that at diagnosis of ALL, patients had a lower BMD than healthy controls [200], although this was not consistently the case [201], and the reduction in BMD during therapy was also replicated [202].

2.2.4 Bone mineral density of patients treated for acute lymphoblastic leukaemia after completion of therapy

One of the challenges of comparing studies assessing BMD of patients who have completed therapy for ALL is the heterogeneity between patient groups arising from differences in treatment regimens. However, the most robust studies, which used an appropriate control group and adjusted for height, found that after completion of chemotherapy, there was no difference in the LSBMD of patients compared with healthy peers [40, 204, 208, 211, 213]. The timing of bone density assessment in these studies ranged from 2 years to 38 years after completion of chemotherapy. However, one large Dutch study by den Hoed et al [209] found that patients who had treatment for ALL had lower height adjusted LSBMD than healthy peers at a median of 16.7 years after cessation of chemotherapy (mean LSSDS -0.3, $p < 0.001$) [209]. It should be noted that in this study the treatment protocols for patients with ALL were not specified, and patients were treated between 1965 and 2003, over which time treatment regimens changed considerably.

The study by Gurney et al [210] was the largest study to look at BMD in long term survivors of childhood ALL. The median age of evaluation of BMD, using QCT, was 31 years, with a median age of leukaemia diagnosis of 5 years. The overall prevalence of patients with a BMD Z-score of ≤ -2 was 5.7%. In a normally distributed healthy population it would be expected that 2.3% of the population would have a Z-score below -2, but there was no statistical analysis in this study to determine if the result was significantly different to the healthy population. Of the 845 patients assessed in this study, 400 had a previous BMD assessment, with a median time between tests of 8.5 years. The mean difference between BMD Z-scores was not statistically significant (Z score -0.086, CI -0.2 to 0.031, $p = 0.15$), but 67% of patients who had an initial BMD Z-score ≤ -2 showed improvement in their

subsequent assessment. Of the 845 patients assessed, 518 (61%) had cranial radiotherapy, and 21 (2.5%) were HSCT recipients.

When change in BMD after cessation of ALL therapy was assessed it was found that on average DXA Z-scores at all sites increased over time [40], and gains in bone mineral content and density were most pronounced in the cohort shortly after completion of therapy. This may suggest that there is a period of time shortly after ALL therapy is completed during which BMD rapidly recovers.

2.2.5 Risk factors for low bone mineral density

In the largest studies to assess risk factors for low BMD, younger age was significantly associated with lower BMD Z-score both at diagnosis of ALL [198, 203] and after cessation of therapy [209]. Low BMI was also found to be an independent risk factor for low BMD in a number of studies [203, 209, 214, 217, 218].

A low BMD at diagnosis has been shown to persist through ALL treatment [198] with larger declines in BMD during treatment found in older patients (age >10 years [198, 203]), those with a lower BMD Z-score at diagnosis [198] and those with a larger dexamethasone area under the curve [198]. Lower albumin levels, which can be caused by the administration of ASP, and older age are associated with a larger dexamethasone area under the curve (reflecting an increased actual body exposure to dexamethasone) [132]. However, there are varying methods for calculating dexamethasone area under the curve, and hence results can vary depending on the methodology [219].

Although older patients had a greater decline in BMD during treatment, after cessation of therapy patients aged ≥ 10 years at diagnosis had significantly greater increases in BMD Z-score from end of therapy to 2 years off therapy [198] compared with those < 10 years of age at diagnosis of ALL.

Factors not associated with a greater decline in BMD were sex, white cell count at diagnosis, B or T lineage, BMI Z-score change, and presence or absence of CNS involvement [198]. Being on a high risk protocol arm was found to be significantly associated with a greater decline in BMD in the Dutch study [203], but this was not replicated in a US study [198]. In the Dutch protocol, the high risk arm contained lower steroid doses than the non-high risk arm, suggesting that factors other than steroid dose are likely to be important in the development of bone morbidity.

Methotrexate has been shown to result in increased osteoclast formation, with increased bone loss [220]. It may be anticipated that higher methotrexate doses would result in lower BMD. However, in long term survivors of ALL treatment methotrexate dose was not found to be associated with lower BMD [210] and a Finnish study found no single chemotherapeutic agent showed an independent relationship with BMD [205].

A genome-wide association study looking at the association between BMD and ALL treatment was reported by Inaba et al [198]. In this study 481,281 SNP genotypes were studied and genotypes were evaluated for associations with BMD Z-score changes from diagnosis to week 120 of maintenance treatment, using significance levels of $p < 1 \times 10^{-4}$. Genomic analysis found the strongest association SNPs with BMD Z-score changes from diagnosis to week 120 was in a SNP in the collagen gene COL11A1 and a SNP in the neural epidermal growth factor-like 1 (NELL1) [198]. These are both genes important in osteogenesis and bone mineralisation. COL11A1 encodes the α -1 chain of type XI collagen, and there is significant phenotypic variation in individuals with COL11A1 mutations [221]. NELL1 is a secreted protein whose expression promotes osteoblast cell differentiation and terminal mineralisation, with inhibition of osteoclast-induced bone resorption. Decreased NELL1 expression leads to skeletal under-mineralisation [222]. However, none of the SNPs reached genome-wide significance ($< 5 \times 10^{-8}$), which may be related to the sample size of the study.

A number of studies found that patients who required cranial or craniospinal irradiation had lower BMD after treatment [209, 210], with female survivors appearing to be more susceptible to craniospinal radiation effects [210]. This is now rarely used as therapy for ALL in the UK, so these results are less applicable to our current patient population.

2.2.6 Fracture risk of patients during and after treatment for acute lymphoblastic leukaemia

Fractures are an important indicator of skeletal fragility, and the significance of diagnosing vertebral fractures and low trauma fractures is increasingly recognised [223]. Vertebral fractures often go undetected as children with vertebral fractures are frequently asymptomatic, and in the past there has been no routine screening of at-risk children [223]. Patients with ALL would be categorised as 'at-risk' due to the use of extensive glucocorticoid therapy during treatment, which preferentially attacks the trabecular-rich spine [74].

The Canadian STOPP study was most robust prospective study to look at vertebral fracture prevalence in young people being treated for ALL. Results have been described in a series of papers authored by Halton et al (2009) [215], Alos et al (2012) [196], Cummings et al (2015) [41] and Ward et al (2018) [216]. In this study 186 patients were recruited for prospective screening for asymptomatic vertebral fractures at baseline, and then annually for 6 years. Fractures were graded using the Genant semi-quantitative method for vertebral morphometry [47]. Data about radiologically confirmed, symptomatic, low trauma non-vertebral fractures was also collected.

Halton (2009) [215] found that in vertebral radiographs taken within 30 days of chemotherapy initiation, 16% of patients had prevalent vertebral fractures. Of these patients (n=29), 52% had one prevalent vertebral fracture, 27% had 2 to 5 fractures and 21% had between 6 and 10 fractures. In patients with vertebral fractures 48% of patients had grade 1 fractures, 31% grade 2 fractures and 21% grade 3 fractures as their highest grade. The majority of fractures occurred in the mid thoracic (T6/T7) and thoraco-lumbar region (T12-L2).

Alos et al [196] described that at 12 months, there were incident vertebral fractures (defined as a new fracture in a previously normal vertebral body, or worsening of an existing VF) in 16% of study patients (n=25) who completed 12 month data collection (n=155). A total of 61 incident VFs were detected. 85% of these (n=52) were in previously normal vertebral bodies. A single VF was found in 52% of affected patients, 28% had 2 to 3 fractures, and 20% had 4 to 10 incident fractures. Again fractures were clustered around the mid-thoracic region and thoraco-lumbar region. At 4 years the study assessed VFs in 136 patients. A total of 105 incident VFs were identified in 38 children in the 4 years following diagnosis [41]. The 4 year cumulative incidence was 26.4%, with an unadjusted VF incident rate of 8.7/100 person-years.

At 6 years 102 patients were assessed for VFs, with results reported by Ward et al (2018) [216]. In the 5th year, 38 patients (37%) had incident VFs, but all of those patients had at least one incident VF in the first 4 years. There were no incident VFs in the 6th year, and 6 year cumulative incidence of VF was found to be 32.5%, [216] with 39% of patients with incident VF reported to be asymptomatic. The final paper also reports the 6 year non-VF cumulative incidence as 23% [216], with an incidence 2.5 times higher than reported in the general population [224]. Non-VFs occurred in 1.6% at

baseline, with peak annual incidences occurring at 2 years (5.4%) and 5 years (4.8%) [216].

Additional smaller studies have also assessed vertebral and non-VF incidence in paediatric patients with ALL. In one study male survivors of childhood ALL were assessed between 10 and 38 years after diagnosis and vertebral compression fractures were found in 20% of survivors [213]. However, in this population 73% of patients had cranial irradiation and 35% of patients had testicular irradiation, potentially limiting the applicability of these results to our patient population.

The Dutch study of 672 patients reported by te Winkel et al assessed cumulative symptomatic fracture incidence during ALL therapy and found similar results to that of Ward et al, with an estimated cumulative incidence of 17.8% at 3 years [203]. This is similar to the study by Rayar et al, which found 18.5% of patients developed symptomatic fractures during ALL treatment [202].

2.2.7 Risk factors associated with increased fracture rate in patients with acute lymphoblastic leukaemia

In the Canadian STOPP study it was found that glucocorticoid exposure and VFs at baseline were significantly associated with increased vertebral and non-VF risk ($p < 0.01$, HR 6.25, CI 3.22-12.15 and $p < 0.01$, HR 4.52, CI 2.01-10.15 respectively) [216]. LSBMD Z-score was found to be significantly reduced in children with prevalent and incident VFs ($p = 0.01$, HR 1.55, CI 1.12-2.13) [41, 196, 215] and this was also found to be the case in patients with symptomatic non-VFs [202, 203, 216].

Decreased age at baseline was also found to be a risk factor for development of incident VFs ($p = 0.01$, HR 1.09, CI 1.02-1.16), but not for non-VFs ($p = 0.27$) [216].

There was no significant difference in age, gender, leukaemia sub-diagnosis, white blood cell count, leukaemia risk category, height, weight, bone age or family history of osteoporosis in likelihood of development of VFs at baseline [215]. Calcium, vitamin D intake and physical activity were not related to VF development, and there was no evidence of an independent effect of puberty on VF risk [215].

This correlates with the results of the study by te Winkel et al, which assessed symptomatic fractures, and found age at diagnosis of ALL, gender, pubertal stage and anthropometry parameters were no different between patients who developed fractures and those who did not [203]. Treatment-

related bone loss was similar in patients with and without fractures, indicating that it is low values of LSBMD at diagnosis and during treatment, rather than treatment related decline, that determines fracture risk in children with ALL [203].

2.2.8 The natural history of vertebral fractures in patients with acute lymphoblastic leukaemia

It is important to recognise the wide spectrum of recovery from fracture-induced vertebral deformity in children with ALL. Some patients have spontaneous vertebral body reshaping with no treatment, whilst others have debilitating back pain with bisphosphonate therapy used to facilitate reshaping [225, 226]. Vertebral body reshaping is a growth-dependent phenomenon that results from bone modelling, and hence can only occur in childhood.

The 6 year results of the STOPP study found that 77% of children with VFs had complete vertebral reshaping (n=34), 18% had incomplete reshaping (n=8) [216] and two patients (4.5%) had no change. However, only 44 children (23.7%) met the inclusion criteria for evaluating reshaping of vertebral bodies, and so the results need to be interpreted with care. Those with incomplete/ absent reshaping were older at diagnosis (median age 8.0 versus 4.8 years) and had more frequent and severe VFs at baseline [216, 226]. The reduction in vertebral reshaping in older patients may be due to lack of remaining linear growth potential for “catch-up” modelling. Vertebral reshaping was not associated with LSBMD Z-score or with change in BMD Z-score from baseline to last visit [216].

2.2.9 Summary

In patients with ALL, studies suggest that BMD is lower at diagnosis of ALL than in healthy peers, and this is more marked in younger patients and those with a low BMI. This is perhaps unsurprising, given the gross infiltration of the bone marrow with proliferating leukaemic cells at the point of diagnosis of leukaemia, and the corresponding increased cytokine activity. BMD appears to reduce or remain low during ALL treatment, particularly in older patients, but subsequently recovers after completion of chemotherapy. The most rapid period of recovery is likely to be shortly after the completion of therapy, with older patients showing greater increases in BMD after cessation of therapy.

There are likely to be genetic factors which have significant influence over BMD in patients with ALL. Thus far there has only been one GWAS, which implicated genes affecting osteogenesis and bone mineralisation.

Ultimately, the relevance of BMD of ALL patients and survivors is to determine if the disease or its treatment has had a detrimental effect on bone fragility, with a resultant increased risk of fractures. The high rate of fractures in paediatric patients with ALL is being increasingly recognised. One study found VFs affected 16% of patients at diagnosis of ALL, with a 6 year cumulative incidence of 32.5% [216]. The critical period for development of VFs appears to be in the first 2 years of chemotherapy with no fractures occurring by 6 years after ALL diagnosis [216]. Peak incidence of VFs was found to be 12 months after diagnosis. Low trauma non-VFs are also more common than in the general population, occurring in about a fifth of patients. It is possible that vertebral bodies are more affected than other areas of the skeleton because vertebral bodies are largely composed of metabolically active trabecular bone, whilst bone turnover in cortical bone is lower and hence less easily affected [227]. The main risk factors for development of vertebral and non-VFs were higher glucocorticoid exposure, presence of VFs at baseline and a lower spine BMD Z-score at baseline.

Although the VF incidence is relatively high, 39% of patients with VFs were asymptomatic. Thus it is imperative to understand the natural history of these fractures, particularly to prevent over-management of patients. It appears that the majority of children with VFs will restore normal vertebral dimensions. However, older patients who are likely to have limited remaining growth potential, or those with more severe vertebral collapse, are at greater risk of persistent deformity.

2.3 Potential therapeutic strategies

From the preceding literature review, it is clear that the bones of young people who are treated for ALL suffer from numerous insults, resulting in many patients suffering with ON and/or increased bone fragility. Despite this knowledge, within the haematology community there is currently no consensus on how to manage these conditions, and an uncertainty about how to optimise the bone health of young patients with ALL [228].

The final part of this chapter focusses on potential therapeutic interventions. For the purpose of this thesis, I have focussed on the use of bisphosphonates for the management of ON in young people with ALL, and the role of vitamin D. These therapeutic interventions were chosen as both have been proposed as potentially beneficial for patients with ON or bone fragility, but their explicit role in young people with ALL remains controversial. The review of the role of vitamin D in children and young people with ALL was conducted as a full systematic review, with planned meta-analysis.

2.3.1 Bisphosphonate therapy in the management of young patients with osteonecrosis and acute lymphoblastic leukaemia

2.3.1.1 Background

Bisphosphonates are widely used in clinical practice to inhibit bone resorption. Their main use is in the management of hypercalcaemia, osteoporosis, osteogenesis imperfecta, metastatic bone disease and Paget disease.

Structurally, bisphosphonates are chemically stable derivatives of inorganic pyrophosphate, which is a naturally occurring compound of 2 phosphate groups linked by esterification [229]. Bisphosphonates inhibit osteoclastic bone resorption by attaching to hydroxyapatite binding sites on bony surface, with a predominance on surfaces undergoing active resorption. When osteoclasts begin to resorb bone that is impregnated with bisphosphonates, the bisphosphonates inhibit hydroxyapatite breakdown, thus suppressing bone resorption [230]. Bisphosphonates also reduce osteoclast activity by decreasing osteoclast progenitor development and recruitment and by promoting osteoclast apoptosis [231]. Moreover, bisphosphonates appear to have a beneficial effect on osteoblasts, with

mouse models finding bisphosphonates prevent osteocyte and osteoblast apoptosis [232].

First generation bisphosphonates are non-nitrogen containing and include agents such as etidronate, clodronate and tiludronate. They have a different mechanism of action to second and third generation bisphosphonates (e.g. pamidronate and zoledronate respectively), which have nitrogen containing side chains. The presence of a nitrogen group increases the bisphosphonates anti-resorptive potency by 10-10,000, with zoledronic acid being 10,000 times more potent than etidronate and 100 times more potent than pamidronate [233].

It has been hypothesised that the suppression of necrotic bone resorption could help in maintaining the spherical shape of the femoral head, allowing revascularisation and preventing femoral head collapse in ON [234] and bisphosphonates have been considered to be a promising medical intervention for osteonecrosis [235]. However, the actual benefit of bisphosphonates in ON is unclear, and this review aimed to answer the following question:

- In a patient with osteonecrosis and childhood acute lymphoblastic leukaemia, what is the evidence for use of bisphosphonates versus no bisphosphonates in reducing pain or improving functional or radiological outcomes?

2.3.1.2 Search Strategy

Primary Search

EMBASE and Medline were searched using the Ovid Medline database (1946 to present) in November 2018. The following terms were used (subject headings=SH): ('osteonecrosis' (SH) OR 'avascular necrosis' OR 'aseptic necrosis') AND ('glucocorticoids' (SH) OR steroids OR leuk*emia) AND ('diphosphonates' (SH) OR 'alendronate' (SH) OR 'bisphosphonates' OR 'pamidronate' OR 'zoledronic acid' OR 'risedronate'). The search was restricted to studies conducted on human beings and limited to publications in English.

Secondary Search

A search of EBM Reviews - Cochrane Database of Systematic Reviews 2005 to June 2018, EBM Reviews - ACP Journal Club 1991 to July 2018, EBM Reviews - Database of Abstracts of Reviews of Effects 2nd Quarter 2018, EBM Reviews - Cochrane Central Register of Controlled Trials July

2018, EBM Reviews - Cochrane Methodology Register 3rd Quarter 2018, EBM Reviews - Health Technology Assessment 3rd Quarter 2018, EBM Reviews - NHS Economic Evaluation Database 2nd Quarter 2018 found no new articles for review.

During the research period weekly reports from Ovid and Embase with any of the above terms in the abstract, title or as a keyword were reviewed and added, and this review was last updated in May 2019.

2.3.1.3 Inclusion criteria

Studies involving patients diagnosed with ALL under 25 years of age, and use of bisphosphonate therapy for the management or prevention of radiologically identified ON.

2.3.1.4 Outcome measures

Outcome measures assessed were:

- Pain
- Development of symptomatic ON
- Functional ability
- Radiological changes of ON

Reported adverse drug reactions and adverse drug events were also considered.

2.3.1.5 Results

524 articles were identified. Only studies looking at the effect of bisphosphonates in osteonecrosis were included, leaving 11 articles. There were only 3 relevant comparative studies described in full text articles [236-238], none of which were relevant to paediatrics. Three conference abstracts of interest were identified [239-241]. Of these, only two included patients who were less than 25 years of age [239, 241], one of which included a randomised control group [239]. There were full text articles of 8 non-comparative studies, of which 5 were relevant to paediatrics [234, 242-245]. The relevant paediatric studies are summarised in Table 13.

Table 13. Studies of bisphosphonate therapy in childhood acute lymphoblastic leukaemia

First author, Country Year of publication	Study design	Study population	Number of patients	Intervention (time of starting after diagnosis of ON)	Duration of intervention	Control	Outcome(s) of interest measured	Results	Additional comments
Agarwala India 2018 [234]	Retrospective case series	Childhood ALL and ON of femoral head Patients age 13-25. Mean age 18.4 years (range 14-24 years)	28	5 mg IV zoledronic acid at baseline then annually, oral alendronate 70mg weekly (not specified)	Mean 50.35 months	n/a	Radiological assessment; pain; Harris hip score	Improvement in pain; improvement in Harris hip scores after start of therapy; radiological collapse in 26%	Patients also received oral calcium 500mg and vitamin D 400IU Prednisolone chemotherapy stopped when ON diagnosed
Bostrom USA 2018 [239]	Retrospective randomized trial	Childhood ALL and developing symptomatic ON Age 10-28 years at time of ALL diagnosis	62 (23 intervention, 39 controls)	Monthly IV pamidromate starting in the first year of therapy (not specified)	12 months	No treatment	Development of symptomatic ON	Incidence of symptomatic ON significantly lower (14% versus 43%) in pamidronate group versus controls	Conference abstract. Only 14 of 39 control patients were developing symptomatic ON compared with all patients in intervention group

First author, Country Year of publication	Study design	Study population	Number of patients	Intervention (time of starting after diagnosis of ON)	Duration of intervention	Control	Outcome(s) of interest measured	Results	Additional comments
Kotecha, Australia 2010 [243]	Observational case series	ALL and ON. Median age (at diagnosis of ALL) 13.5 years (range 5.42-16.58 year)	17 6/17 intermediate risk, of which 3 changed to high risk group 3/17 treated from start as high risk 8/17 treated conservatively	Intermediate risk -oral alendronate 70mg/week of High risk -monthly pamidronate infusions 30-65mg/m ² (not specified)	Alendronate Median 11.5 months Pamidronate Median 5 months	Conservative therapy: calcium, vitamin D, dietetic review and physiotherapy	Range of movement; function pain; radiological assessment (MRI)	Conservative treatment: 7/8: no improvement. 1/8: resolution of symptoms. Alendronate: 3/6: no improvement, changed to pamidronate. 3/6: improvement in pain. 2/3: improvement in function and range of movement. Pamidronate: 6/6: improvement in clinical outcomes. 5/6: improvement in pain. All patients had reduction in total volume of ON. No difference in rate of healing between groups	Allocated conservative or medical therapy depending on clinician risk categorisation, hence groups non-comparable. Non-uniformity of pamidronate dosing and schedule
Leblicq Canada 2013 [244]	Retrospective case series	Childhood ALL and symptomatic ON. Median age (at diagnosis of ALL): 11 years (range 2.7-16.6 years)	17 (14 given pamidronate, 3 controls)	Pamidronate: initial doses: day 1: 0.5mg/kg day 1, day 2+3: 1mg/kg/day, subsequently, 1mg/kg/day for 3 days every 4 months. (not specified)	Median 6.3 months (range 1-26.7). Pamidronate given until 6 pain-free months or end of therapy, whichever later	No treatment	Pain score; motor function score; radiological progression	11/13: resolution of bone pain; 9/12: improvement in motor function; 9/14: ON lesions stable/ improved, 5/14 deteriorated In untreated patients: no improvement in pain score; 2/3 improved motor function	3 patients not given pamidronate due to clinician choice. All patients had calcium and vitamin D supplementation, and physiotherapy. All patients stopped steroids at time of ON diagnosis

First author, Country Year of publication	Study design	Study population	Number of patients	Intervention (time of starting after diagnosis of ON)	Duration of intervention	Control	Outcome(s) of interest measured	Results	Additional comments
Nguyen Australia 2006 [245]	Case series	Childhood ALL and ON. Median age (diagnosis of ALL) 14.3 (range 10.8-17 years)	6	Pamidronate 1mg/kg every 2 months (Mean 9.2 months, range 1-18 months)	2 years.	n/a	Mobility; pain; radiological assessment	4/6: reduction of pain and increased mobility in first year. In second year of treatment: 3 patients unchanged, 2 worsened; no improvement in MRI of affected areas	4 patients had cessation of steroids. The two patients who continued to be given dexamethasone had no clinical improvement
Padhye Australia 2013 [242]	Retrospective chart review	Young people with ON and a haematological disorder. Median age (start of study): 13 year (range 7.84-14.52 year)	20 (12 patients with ALL)	Zoledronic acid 0.025mg/kg/dose IV every 12 weeks. (median 1.40 years, range 0.25-4.54 years)	Median 13 months.	n/a	Pain score; radiographic assessment	Pain reduction in all patients; 8/9 with hip predominance: radiological progression 3/8 with knee predominance: radiological progression	Variation in primary diagnosis treatment protocol. 8 patients had stem cell transplant. Patients also given 400IU vitamin D and calcium if low dietary intake
Shaw USA 2013 [241]	Case series	Childhood ALL and ON Age not given	4	Pamidronate every 2 months (not specified)	6 months.	n/a	Pain; range of movement; ambulation; radiological assessment	4/4: improved clinical outcomes, no radiographic or MRI improvement	Conference abstract. Insufficient details regarding study design, methodology and statistical analysis to draw conclusions from results

Table 13 highlights the significant heterogeneity between studies. There is only one randomised study, which was described in an abstract [239]. The studies all had small sample sizes, with variable duration, type and dose of treatment, and different intervals between onset of ON and use of bisphosphonates. All of these may result in considerable variation in any efficacious effect of bisphosphonates. There were also considerable differences in study design, with one study categorizing patients into low, medium and high risk, and allocating treatment accordingly [243]. It is known that the size of necrotic lesion, and involvement of articular surface is particularly important in predicting outcome [118], so there is an inherent difficulty in grouping all patients with ON together. The majority of patients in the studies were diagnosed with ALL, but one study also included patients with lymphoma (n=6) and benign haematological disorders (n=2) [242]. Patients both between and within studies were managed with different ALL treatment regimens, with a number of patients receiving cranial or total body irradiation, or stem cell transplantation (which is usually conditioned with total body irradiation). These differences could also significantly alter the progression of osteonecrotic lesions. The general management of patients with ON also varied between and within studies, particularly with regards to cessation of steroids and use of vitamin D and calcium supplementation. When looking at outcome measures it should be noted that there are no universal scales for outcome measures such as pain, mobility or level of function, resulting in difficulties comparing studies. Due to the above issues, no meta-analysis was possible and a narrative summary of results is provided below.

2.3.1.5.1 Pain

The results suggest that pain was reduced in all or most patients with ON and ALL who were given pamidronate or zoledronate therapy [234, 241-243]. Results from 1 study suggests oral alendronate therapy is less efficacious in reducing pain [243]. The largest case series of patients who received zoledronic acid then alendronate was described by Agarwala et al, and found that mean pain score reduced from 5.82 at the start of therapy to 2.72 in a mean duration of 5.2 weeks [234]. The pain score was statistically lower than at presentation at all time points following initiation of bisphosphonates, regardless of Ficat-Arlet stage of ON. There was also a corresponding reduction in mean analgesic requirement [234]. It is known that a significant number of patients with ON have spontaneous resolution, with resultant reduction in pain, and it is unclear to what extent pain would

spontaneously resolve in these non-randomised studies. Despite this, the speed of improvement in pain does suggest a positive effect of bisphosphonate therapy. A few studies compared patients who were given bisphosphonates with those who were not. They found that in the patients who were not treated with bisphosphonates, within the study follow up time, there was no, or minimal reduction in pain [243, 244]. However, comparator groups were not clinically equivalent to treated groups in either of these studies.

2.3.1.5.2 Development of symptomatic ON

Only one study looked at the use of prophylactic bisphosphonate therapy in reducing development of symptomatic ON. This was reported in an abstract by Bostrom et al, and was a retrospective study using prophylactic pamidronate therapy within the first year of therapy for ALL patients aged between 10 and 28 years who were developing symptomatic ON [239]. In the 23 patients who were given prophylactic pamidronate, the incidence of development of symptomatic ON was significantly lower than that in the control group (14% versus 43%, $p=0.049$). Within the control group of 39 patients, only 14 were developing symptomatic ON at the start of the study. Although this means the control group is not directly comparable with the treatment group, it would be expected that the direction of bias would be towards an increase in development of symptomatic ON in the treatment arm, yet the opposite was seen. The method of selecting patients who were 'developing symptomatic ON' was not defined in this abstract, and the study is described as retrospective, making results less robust than those of a prospective randomised control trial [239].

2.3.1.5.3 Functional ability of patients

The functional outcome of patients was assessed in 5 studies. The study by Leblicq et al found 9 of 12 patients treated with bisphosphonates had an improvement in motor function [244]. This is similar to the findings by Nguyen et al where 4 of 6 patients had improved mobility at 1 year [245], and in the 4 patients treated in the study reported by Shaw et al, all patients had increased range of movement and ambulation [241]. In the study by Kotecha et al, all 6 high risk patients who were given pamidronate had improvement in function and range of movement, whilst in the intermediate risk group who were given alendronate, only 2 of 6 patients had improvement in function [243]. In the low risk group who were treated conservatively only 1 of 8 patients showed improvement in function and range of movement [243]. The study reported by Agarwala et al found that

there was a statistical improvement in Harris Hip Score for all stages of ON after treatment with bisphosphonates, compared with the start of the study [234]. The Harris Hip Score is a clinician-based outcome measure and covers the domains of pain, function, absence of deformity and range of motion. However, it should be noted that the Harris Hip Score was developed in 1969 for the assessment of the results of hip surgery in an adult population [246], which calls into question its use in this population. Again, the lack of a control group in these all of these studies limits the strength of these findings.

2.3.1.5.4 Radiological changes of ON

As previously described, the natural history of osteonecrosis is far from clear, particularly in the paediatric population. We are aware that some patients will have reversal of osteonecrotic lesions, whilst other will have lesions that progress and result in joint collapse [131, 141]. In the study by Kotecha et al, it was found that regardless of therapeutic intervention, all patients showed a reduction in total volume of ON with time, with no statistical difference between patients treated with pamidronate and alendronate [243]. Nguyen et al found no improvement in MRI features of ON in patients treated with pamidronate [245] and this was also found to be the case in the report by Shaw et al [241]. The study by Agarwala et al found radiological progression in 38% of hips (n=13) at a median follow-up time of 50 months, with radiological collapse in 26% of hips (n=9) [234].

There are few relevant prospective natural history studies, and the natural history may vary depending on size/ site of lesion at the start of the period of observation. In the earlier literature review the SJCRH study was described, which found that in 130 patients with asymptomatic ON, 11% had lesions that resolved, whilst in 26% the grade of lesions worsened [132].

2.3.1.5.5 Adverse effects

In all studies, definitions and methods of data collections for adverse events were rarely published, and therefore adverse events were likely to be detected opportunistically.

The most commonly described adverse event was the classical acute phase reaction to bisphosphonates, which is a non-specific physiological and biochemical reaction that typically includes fever and mild myalgias and arthralgias [247, 248]. Other adverse events that have been reported in studies assessing safety of bisphosphonate therapy include hypocalcaemia, which typically affects patients with suboptimal vitamin D or calcium intake,

and gastric irritation secondary to oral bisphosphonate use [249]. Osteonecrosis of the jaw, atrial fibrillation and atypical femoral fractures are rare complications of bisphosphonate therapy, but these have never been described in paediatric patients [249].

Pamidronate was the most commonly used bisphosphonate, used in the studies reported by Bostrom [239], Kotecha [243], Leblcq [244], Nguyen [245] and Shaw [241]. The acute phase reaction was reported in 50% of patients in the study by Lebliq et al [244], and by 'most patients' in the study by Nguyen et al [245], with 'no untoward side-effects from pamidronate' reported in the abstract by Bostrom et al [239]. The studies reported by Kotecha et al and Shaw et al made no mention of any side-effects of pamidronate treatment.

Zolendronic acid was used in the studies reported by Padhye and Agarwala [234, 242]. An acute phase reaction was reported in 10% (n=2) of patients included in the study by Agarwala et al [234] whilst in the study by Padhye et al, 35% of patients (n=7) experienced an acute phase reaction after the first dose [242].

After the first dose of intravenous bisphosphonate, subsequent doses were uneventful, with no patients experiencing clinically significant hypocalcaemia or bisphosphonate related osteonecrosis of the jaw.

Two studies used oral alendronate therapy. One of 6 patients (17%) who used alendronate in the study described by Kotecha et al reported recurrent gastrointestinal upset whilst on treatment [243]. In the study by Agarawala et al, mild dyspeptic symptoms were reported in 3 patients (15%), which resolved within one month of starting therapy [234].

2.3.1.6 Conclusion

In conclusion, there are methodological flaws in all of the studies described, but in selected cases there is limited evidence that pamidronate and zolendronate may be beneficial for pain management. There is also a suggestion that functional ability of patients improves with use of pamidronate or zolendronate, but there is no clear evidence to suggest that bisphosphonates alter radiological progression of osteonecrosis in childhood. In these small studies, bisphosphonates appeared to be well tolerated, with an acute phase reaction after the first dose of pamidronate or zolendronic acid the most common adverse event. Individual patient characteristics would need to be considered before use, and the optimum treatment regimen is unclear.

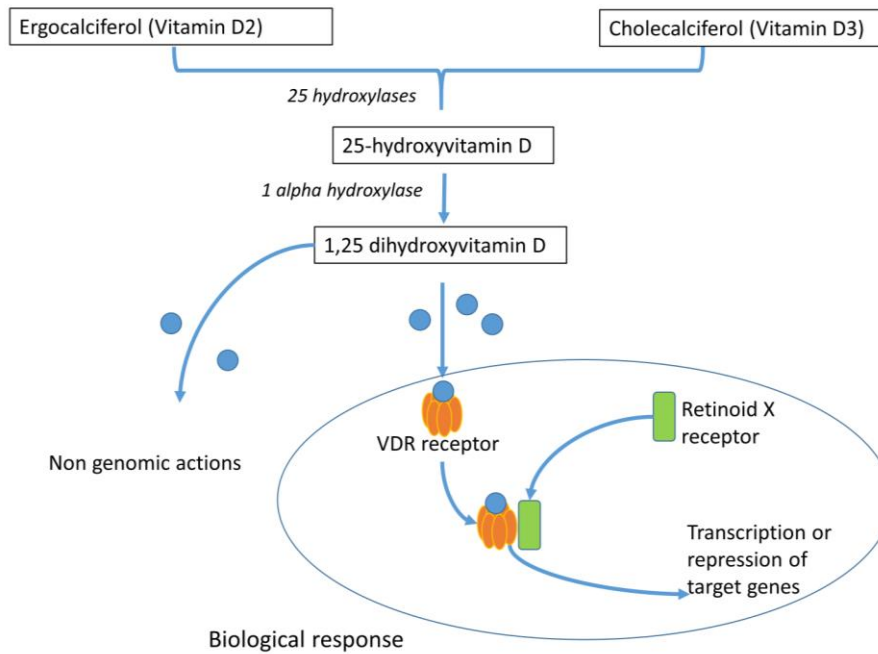
2.3.2 Efficacy and safety of vitamin D in children and young people with acute lymphoblastic leukaemia: a systematic review

2.3.2.1 Background

Vitamin D plays a pivotal role in calcium and phosphate metabolism, and is an important variable in bone health. It has also been shown to have anti-cancer and immunomodulatory effects: in some cancers vitamin D and its derivatives inhibit proliferation, induce apoptosis, reduce angiogenesis and sensitise cells to chemotherapy [250]. In a meta-analysis of adult patients with any haematological malignancy, it was found that low serum 25-hydroxyvitamin D (25OHD) levels were significantly correlated with reduced overall survival and relapse free survival time [251]. However, this meta-analysis did not include paediatric patients, who are affected by different haematological malignancies compared with adult patients [252].

The endocrine regulation of bone metabolism has been described in the introductory chapter, highlighting the importance of Vitamin D in the maintenance of calcium homeostasis. Vitamin D exists as 2 forms: ergocalciferol (vitamin D₂), and cholecalciferol (vitamin D₃). Following intestinal absorption both are metabolised to 25OHD at the liver, which undergoes subsequent conversion in the kidney and other tissues possessing 1- α hydroxylase activity to 1, 25-dihydroxycholecalciferol (1,25(OH)₂D), the biologically active form. Calcitriol is the synthetic physiologically active analogue of 1,25(OH)₂D, specifically the vitamin D₃ form. Typically 1,25(OH)₂D will bind to the nuclear vitamin D receptor (VDR) to induce a conformational change in the protein, which permits binding to the retinoid X receptor. The heterodimer then acts as a transcription factor, causing transcription or repression of target genes [250] (see Figure 7).

The VDR is expressed in at least 30 different target tissues including bone, kidney, blood, breast, prostate, gut, activated B and T-lymphocytes, monocytes and keratinocytes [253, 254]. In some cells, such as intestinal cells, a membrane receptor for 1,25(OH)₂D has also been shown to exist, but it is unclear if this receptor is present in other types of cells [255, 256]. VDRs have also been found in breast cancer [257], human melanoma [258], neuroblastoma [259], prostate cancer [260] and myeloid leukaemia cells [261]. However, in a small study of 4 ALL cell lines, no ALL cells had detectable levels of VDR [262], although *in vitro* calcitriol was found to inhibit proliferation and promote apoptosis of activated B cells [262].

Figure 7. Vitamin D metabolism

Vitamin D has both genomic and non-genomic effects, and facilitates active calcium and phosphate transport in the intestine, resulting in a net increase in serum calcium and phosphate [263]. It also acts to increase reabsorption of calcium in the distal renal tubules and stimulates phosphate reabsorption at the proximal tubule [264]. In bone, 1,25(OH)₂D has both anabolic and catabolic actions, but appears to facilitate bone formation at physiologically optimal concentrations, while higher levels promote resorption and limit mineralisation to sculpt bone [264]. In vitamin D deficient states there is a corresponding increase in parathyroid hormone (PTH), which can result in pathological resorption in bones and increased bone fragility. Vitamin D also maintains phosphate homeostasis via its interaction with FGF23, a key phosphate regulator. 1,25(OH)₂D induces the release of FGF23 from bone, resulting in phosphaturia, a process which is independently stimulated by high phosphate levels.

The most widely used preparation for treatment of vitamin D deficiency is cholecalciferol. Activated vitamin D preparations, such as calcitriol or alfacalcidol, are not generally used for the treatment of simple vitamin D deficiency due to the risk of serious adverse effects, including hypercalcaemia [265].

Optimal vitamin D levels in blood are highly debated. Current UK practice, as recommended by the British Paediatric and Adolescent Bone Group, defines a blood level of 25OHD <10ng/ml (25nmol/L) as deficient, with insufficiency defined as between 10-20ng/ml (25-50 nmol/l) [265]. The Endocrine Society defines vitamin D deficiency as a 25-OHD level below 20ng/ml (50nmol/l), and vitamin D insufficiency is defined as a 25OHD level of 21-29ng/ml (52.5-72.5nmol/l) [266]. These cut-off values were set with regards to prevention of rickets and/ or symptomatic osteomalacia, however it should be recognised that in determining risk of development of rickets, other crucial factors need to be taken into consideration, including serum phosphate and dietary intake of calcium, both of which can affect serum PTH. The prevalence of vitamin D deficiency varies considerably depending on the geographic latitude of the population studied, time spent outdoors, fortification of food, age of the population, ethnicity and time of year samples are obtained [267, 268]. The seasonality of vitamin D deficiency is illustrated by a study which reported that between January and March 40% of UK children aged between 11-18 years have a 25OHD level below 25nmol, which falls to 13% between July and September [269].

At present, there is no consensus on how best to manage patients who have newly diagnosed ALL with concurrent vitamin D deficiency, and the benefits and risks of doing so are unclear. This review aims to determine if treatment with vitamin D provides any evidence of clinical benefit or holds any specific risks in this vulnerable patient population.

2.3.2.2 Methods

This review was undertaken following a pre-specified protocol registered on PROSPERO (the international prospective register of systematic reviews): CRD42018092553 April 2018 [270]. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines were also followed [271].

2.3.2.3 Inclusion criteria

Studies involving patients under 25 years of age with ALL with low levels of vitamin D (defined as vitamin D deficient with levels of 25OHD < 10ng/ml (<25nmol/L) or vitamin D insufficient with levels of 25OHD 10-20ng/ml (25-50nmol/L)), treated with any dosing schedule of cholecalciferol, ergocalciferol or calcitriol during treatment for ALL.

Randomised and non-randomised controlled trials were assessed for therapeutic efficacy. Cohort, case control studies and case reports, as well

as randomised and non-randomised controlled trials were included for safety analysis.

2.3.2.4 Exclusion criteria

Patients who received haematopoietic stem cell transplant as treatment for ALL were excluded, due to previous chemotherapy exposure and significant differences in treatment received by patients.

2.3.2.5 Outcome measures

Primary outcome(s)

1. Bone health:
 - a. Prevalence of low impact fractures
 - b. Prevalence of osteonecrosis
2. Acute lymphoblastic leukaemia:
 - a. 1, 2, 3, 5, >5 year survival estimates and median survival time from diagnosis of ALL

Secondary outcome(s)

1. Bone health:
 - a. Bone mineral density
 - b. Calcium, phosphate, PTH levels
 - c. Vitamin D levels
2. Acute lymphoblastic leukaemia:
 - a. Cumulative incidence of relapse
 - b. Response to treatment (as defined by bone marrow minimal residual disease at end of induction chemotherapy)

Reported adverse drug reactions and adverse drug events were also considered.

2.3.2.6 Search methods for identification of studies

Database searches of Embase (1996-2018), Medline (1996-2018), The Cochrane library and Web of Science were undertaken with the following search strategy:

(Leukemia, lymphoid (MESH term) OR leuk?emia (keyword))

AND

(Vitamin D (MESH term) OR Vitamin D Deficiency (MESH term) OR vitamin D (keyword) OR c?olecalciferol (keyword) OR ergocalciferol (keyword) OR calcitriol (keyword))

Reference lists of identified articles and key review articles, abstracts from major conferences and hand searches of journals that comprise the most frequent venues for publications in this area were included.

Searches were performed without language restrictions and attempts were made to obtain a translated copy where possible. Grey literature was also searched.

During the research period weekly reports from Ovid and Embase with any of the above terms in the abstract, title or as a keyword were reviewed and added, and this review was last updated in May 2019.

2.3.2.7 Study selection

Study selection and data extraction was conducted using the following method:

Two reviewers independently assessed the title and abstract of all studies for possible inclusion. Inclusion or exclusion was verified by assessment of the full text of potentially included studies.

Discrepancies between reviewers were resolved by discussion. Unresolved discrepancies were referred to an independent assessor.

2.3.2.8 Data extraction and management

Data were extracted using a standardised form (Appendix 2) which was independently checked by the second reviewer. The author(s) of the paper were contacted when additional information was required, including information on methodological criteria. If no further information was available the criteria was rated as 'unclear'.

The quality of randomised studies was assessed by the Cochrane risk of bias [272] to assess adequacy of methods for sequence generation, concealment of allocation, completeness of outcome data or handling of incomplete outcome data, and blinding of assessors.

Risk of bias in non-randomised studies was assessed using the ROBINS-I (risk of bias in non-randomised studies of interventions) [273].

The Loke method was used to assess quality of studies investigating adverse events [274].

Extracted data was checked for agreement between review authors, and disagreements in methodological assessments were resolved by consulting a third review author, with consensus reached through discussion.

2.3.2.9 Data synthesis

A meta-analysis of survival outcomes was not possible due to the lack of appropriate studies, and a narrative summary undertaken instead. A narrative synthesis was undertaken for the safety analysis, including reporting of adverse events/ reactions occurring within single arm studies. Due to insufficient data, we were unable to perform random effects meta-analysis of logit-transformed proportions experiencing that adverse event/ reaction.

2.3.2.10 Analysis of subgroups or subsets

Subgroup analyses according to age, sex, timing of therapy and vitamin D level at diagnosis was not possible due to lack of sufficient data. Assessment of evidence of between group interactions and qualitative assessment of differences between groups was undertaken. As there were insufficient studies, meta-regression was not performed. Due to lack of sufficient high quality data we were unable to perform a sensitivity analysis, based on risk of bias and study design.

2.3.2.11 Results

We identified 1939 unique articles, of which 22 were eligible for full text review. Of these only 5 studies were found to be appropriate for full review (Figure 8). Only 2 studies were randomised [275, 276] with 3 cohort studies reviewed [277-279]. Indications for exclusion of articles is summarised in Figure 8. Tables 14 and 15 outline the characteristics of the studies included in the subsequent analysis.

One randomised study [275] and one cohort study [278] treated all patients with the vitamin D supplements, regardless of vitamin D status, and the other two cohort studies treated all patients, but with varying doses of cholecalciferol depending on vitamin D status [277, 279]. Although they do not completely fulfil the inclusion criteria for study selection, these studies were selected for inclusion due to their value for the safety analysis, and due to the high prevalence of vitamin D deficiency in the population [269]. The cohort study by Claar et al was only published as an abstract, and communication with the author allowed further details to be obtained [279].

Figure 8. Study flow diagram of search and selection process

Due to the variability between studies, the results were not able to be pooled for any of the outcome measures assessed.

Table 14. Characteristics of studies included for analysis: randomised studies

First author, Country Year of publication	Study design	Patient population	Number of patients	Definition of vitamin D deficiency/ insufficiency (ng/ml)*	Median age of patients, years, (range)	Intervention	Timing of therapy	Control	Outcome(s) of interest	Timing of measurements	Length of follow up	Safety outcomes
Orgel, USA 2017 [276]	RCT	Newly diagnosed ALL, aged 10-21 years with vitamin D insufficiency	29	Insufficiency: <30ng/mL	15.2 (11-19)	Cholecalciferol 2000iu, calcium citrate 1000mg, daily. After interim analysis, 3 doses of cholecalciferol 100,000IU approx 2 monthly, calcium carbonate 400mg BD	Treatment after end of induction	Placebo initially. After interim analysis control was standard of care	Vitamin D levels Volumetric BMD Bone structure and geometry Body composition	Vitamin D levels measured at end of induction, start of interim maintenance, start of DI, end of DI /end of study. BMD and body composition assessed at end of induction and end of DI	Median 6.7 months (range 5.5-8.7 months)	Hypervitaminosis Hypercalcaemia Nephrolithiasis Renal insufficiency Transaminitis
Diaz, Chile 2008 [275]	RCT	Newly diagnosed ALL, Tanner stage 1	16	n/a	5, (1.7-11.5)	Weight <30kg: calcitriol 0.25 micrograms/day Weight > 30kg: calcitriol 0.5 micrograms/ day	Treatment after end of induction	No calcitriol therapy	BMD	BMD at baseline and at 1 year	1 year	Plasma and urinary calcium

*conversion factor for 25 hydroxyvitamin D: 1ng/ml=0.4nmol/l

Table 15. Characteristics of studies included for analysis: cohort studies

First author, Country Year of publication	Study design	Patient population	Number of patients	Definition of vitamin D deficiency/ insufficiency (ng/ml)*	Median age of patients, years, (range)	Intervention	Timing of therapy	Outcome(s) of interest	Timing of measurements	Length of follow up	Safety outcomes
Young USA 2018 [277]	Retrospective cohort	Newly diagnosed ALL patients. Repletion dose of vitamin D for patients with 25-OHD level < 30ng/mL, maintenance dose for all patients with vitamin D sufficiency	69	Deficient: <20ng/mL Insufficient: 20- <30ng/mL	6.7, (0.25-32)	Age <12m: Repletion dose: Cholecalciferol 1000iu OD. Maintenance dose: Cholecalciferol 400iu OD Age 1-5 years: Repletion dose: Cholecalciferol 50,000iu weekly. Maintenance dose: Cholecalciferol 5,000iu weekly or 800iu OD Age>5yrs:Repletion dose: Cholecalciferol 50,000iu weekly. Maintenance dose: 1,000iu OD or 10,000iu weekly	Not documented	Vitamin D levels	At diagnosis and 3 monthly	Median 10.5 months (range 2-22 months)	High vitamin D levels Hypercalcemia Nephrolithiasis

First author, Country Year of publication	Study design	Patient population	Number of patients	Definition of vitamin D deficiency/ insufficiency (ng/ml)*	Median age of patients, years, (range)	Intervention	Timing of therapy	Outcome(s) of interest	Timing of measurements	Length of follow up	Safety outcomes
Demirsoy, Turkey 2017 [278]	Prospective cohort	Newly diagnosed ALL patients.	34 patients, of these 11 had BMD measured	Deficient: <20ng/mL	3.67, (1.28-17.83)	Cholecalciferol 400IU-600IU OD Age <8 years: Calcium carbonate 500mg OD Age >8 years: Calcium carbonate 1000mg OD	Not documented	Vitamin D, calcium, magnesium PTH, ALP Patients >5 years of age: BMD	First week of induction and at completion of re-induction therapy	8 months	Hypercalcaemia Symptomatic nephrolithiasis Symptomatic bone fractures
Claar, USA 2014 [279] (abstract only)	Prospective cohort	Paediatric ALL patients. Patients treated as per algorithm depending on vitamin D status	43	Deficient <20ng/mL Insufficient 21-29ng/mL Sufficient >30 mg/mL**	Not documented	Vitamin D deficient: Cholecalciferol 2000iu OD Vitamin D insufficient: Cholecalciferol 1000iu/day ***	Not documented	Vitamin D sufficiency	Not documented	8-12 weeks	Not documented

*conversion factor for 25 hydroxyvitamin D: 1ng/ml=0.4nmol/l

**Unpublished data, received upon e-mail correspondence with author.

*** full details of Claar et al Vitamin D supplementation guidelines (received upon correspondence with author)

Vit D deficient:cholecalciferol 2000IU/day, recheck levels within 6-8 weeks, if sufficient treat as per sufficient guideline. If remains deficient, increase to 4000IU/day if over 1 yr (refer to endo if age <1yr)

Vit D insufficient: 1000IU/day, recheck levels within 6-8 weeks, if sufficient treat as per sufficient guideline. If remains insufficient: increase to 2000IU/day, recheck in 6-8 weeks

Vit D sufficient: age 0-1yr: 400IU/day, age >1yr: 600-1000IU/day

OD: once daily, PTH: parathyroid hormone, ALP: alkaline phosphatase, BMD: bone mineral density

2.3.2.11.1 Risk of bias assessment

Tables 16 and 17 summarise the risk of bias assessments for the randomised studies and cohort studies respectively. Judgement was made as per the Cochrane risk of bias assessment tool for the randomised controlled studies, and using the ROBINS-I for the non-randomised studies. Only one study, the randomised study by Orgel et al, was found to have an overall low risk of bias in all domains- despite lack of blinding the objective methods of assessment minimises risk of bias [276]. Although the study by Diaz et al had a high risk of bias, the magnitude of bias is likely to be small due to the objective nature of outcome measures, although the confidence in the estimate is reduced [275]. The ROBINS-I identified serious risk of bias in all of the cohort studies assessed, predominantly due to the lack of use of methods to control for confounding.

Table 16. Risk of bias for randomised controlled trials.

Study author, year	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective reporting	Other sources of bias
Orgel, 2017 [276]	Low risk	Low risk	Low risk- no blinding but outcome not likely to be influenced by lack of blinding	Low risk- no blinding but outcome not likely to be influenced by lack of blinding	Low risk	Unclear risk- no protocol available	Low risk
Diaz, 2008 [275]	High risk	High risk	Low risk- no blinding but outcome not likely to be influenced by lack of blinding	Low risk- no blinding but outcome not likely to be influenced by lack of blinding	Low risk	Unclear risk- no protocol available	Low risk

Table 17. Risk of bias for non-randomised studies

First Author, Year	Bias due to confounding	Bias due to participant selection	Bias due to measurement of outcomes or interventions	Bias due to deviations from intended interventions	Bias due to missing data	Bias due to measurement of outcomes	Bias due to selection of reported results	Overall Risk of Bias
Demirsoy, 2017 [278]	Serious	Low	Low	No information	No information- no protocol	Low	No information- no protocol	Serious
Young, 2018 [277]	Serious	Low	Low	No information	No information- no protocol	Low	Risk of selective reporting	Serious
Claar, 2014 [279]	Serious	No information	Low	Yes (results of adherent patients presented)	No information	Low	Serious- favours the intervention	Serious

2.3.2.11.2 Adherence

Adherence to medication was only addressed fully in one study, which highlighted the likelihood of low levels of adherence for prescribed medication. This study, reported by Orgel et al, was initially opened as a double-blinded, placebo controlled prospective trial testing a daily combination of vitamin D3 and calcium citrate [276]. At interim analysis after one-third of target accrual only 23% of patients were adherent by report and tablet count, and a median of 7% of doses were delivered. It was subsequently amended to an open-label trial testing directly observed therapy of high dose vitamin D3 (100,000IU) administered in clinic at the start of each chemotherapy phase, and calcium supplementation was changed to a flavoured, chewable calcium carbonate. With this alteration there was 100% adherence to vitamin D3 and 61% adherence to taking $\geq 75\%$ of prescribed calcium carbonate. In the abstract presented by Claar et al it was reported that 71% of patients were adherent to supplementation, but no further details were given regarding methods of assessing adherence [279].

2.3.2.11.3 Primary outcomes

1a: Prevalence of low impact fractures

There were no studies with the outcome of prevalence of low impact fractures.

1b: Prevalence of osteonecrosis

There were no studies assessing impact of vitamin D therapy on prevalence of osteonecrosis.

2a: 1, 2, 3, 5, >5 years and median survival from diagnosis of ALL

There were no studies assessing impact of vitamin D therapy on survival after diagnosis of ALL.

2.3.2.11.4 Secondary outcomes

1a: Bone mineral density

There were 2 small randomised studies [275, 276] looking at BMD following vitamin D supplementation, with considerable variability between the studies. Neither study found an overall difference in final BMD between groups who did and did not have vitamin D supplementation. The highest quality randomised study was that by Orgel et al, in which patients aged over 10 years with newly diagnosed ALL and with a vitamin D level of less than

30ng/ml were treated with cholecalciferol after completion of induction chemotherapy [276]. Volumetric bone density was assessed using QCT, and no significant differences were observed for trabecular volumetric BMD of the lumbar spine, cortical volumetric BMD of the femur, or bone structure and geometry between randomised groups [276]. The study by Diaz et al assessed pre-pubertal patients with newly diagnosed ALL who were randomised to calcitriol treatment or no treatment after completion of induction therapy. DXA imaging was used to assess LSBMD, hip BMD, total body BMD and total body mineral content, with no difference in BMD found between groups [275]. In a subset analysis, with no *a priori* description of planned analysis, a correlation study reported a greater LSBMD increment in the children with lower initial BMD in the calcitriol group.

In the cohort study by Demirsoy et al, 11 patients with newly diagnosed ALL had DXA imaging within a week of starting induction therapy and then approximately 8 months later. Despite low dose cholecalciferol and calcium supplementation in all patients there was a significant reduction in total body BMD Z-score, TBLH and L1 to L4 BMD Z-score [278]. When final BMD was compared with that of ALL survivors who were 8 to 24 months post-diagnosis and who had not received supplementation, the BMD was lower in the group who had not received supplementation. However, this was a historic control group, with BMD measured at a wide range of time-points post initiation of treatment. Patients in this historic control group were also treated with different chemotherapy protocols, one of which included the administration of prophylactic radiotherapy in the medium risk group, making the 2 groups essentially incomparable.

1b: Calcium, phosphate, parathyroid hormone levels

The study by Orgel et al assessed changes in calcium, phosphate and PTH after vitamin D supplementation in young people with ALL, and found that after cholecalciferol therapy there were no significant differences in corrected calcium, phosphate or PTH levels between treatment and control groups [276].

A small increase in calcium was reported in one cohort study which provided patients with calcium supplementation as well as low dose vitamin D supplementation, with a reported increase in median calcium level from 9.0 to 9.3mg/dL ($p=0.024$) [278].

1c: Vitamin D levels

All of the 4 studies which assessed vitamin D levels found increases after supplementation. Reported results from studies are presented in the Table 18 below. Specific results were not reported in the abstract by Claar et al, but it was reported that with supplementation 92% of adherent patients achieved vitamin D sufficiency within 8-12 weeks [279].

The study by Orgel et al was the only randomised study assessing changes in vitamin D level after supplementation, and found there was a significant increase in vitamin D level after directly observed cholecalciferol treatment [276].

Table 18. Changes in vitamin D level after supplementation

Author, year	Baseline median vitamin D level (ng/mL)	End of study median vitamin D level (mg/mL)	P value	Confidence intervals
Young, 2018 [277]	24.7	47.8	Not reported	Not reported
Orgel, 2017 [276]	19.45	26.5	0.026	Not reported
Demirsoy, 2017 [278]	17.9	23.5	0.01	Not reported

As seasonality will clearly have an effect on vitamin D levels, the papers were assessed to determine when each study was conducted to help ascertain impact on vitamin D levels. In the paper by Young et al, patient recruitment started in November 2014, with 35 patients recruited in winter and 34 recruited in summer [277]. However, 53 patients ended the study in summer, compared with 16 who ended the study in winter. This could potentially result in bias towards an increase in end of study median Vitamin D levels. In the study by Orgel et al, patients were enrolled between May 2011 and November 2014, with a median length of follow up of 6.7 months (range 5.5-8.7 months) [276] but numbers of patients recruited in different seasons was not documented. If more patients ended the study in summer this could result in bias towards an increase in end of study median vitamin D levels, but without a full breakdown of patient start and end dates this was not possible to determine.

The timing of recruitment of de novo ALL patients was not clearly documented in the study by Demirsoy et al or in the abstract by Claar et al.

2a: Rate of relapse of ALL

There were no studies assessing impact of vitamin D on rate of relapse of ALL

2b: ALL response to treatment

There were no studies assessing impact of vitamin D on ALL response to treatment.

2.3.2.11.5 Safety analysis

In the 5 studies included within the safety analysis comprising a total of 191 patients, there were no reported adverse events as a consequence of vitamin D supplementation other than supra-therapeutic, but non-toxic, levels of vitamin D. The Loke method has been used for quality assessment for the reporting of adverse events (Table 19). It can be seen that definitions and methods of data collections for adverse events were rarely published, implying adverse events were likely to be detected opportunistically.

The main adverse events that may be anticipated are hypercalcaemia, nephrolithiasis and supra-therapeutic levels of vitamin D. Of these, levels of vitamin D over 150ng/ml were reported in 4 of 69 patients (6%) in the study by Young et al [277]. This study also reported 8 episodes of nephrolithiasis in 7 patients (10%), none of which were in the setting of elevated 25OHD levels or hypercalcemia. There were no toxicity or adverse events attributed to cholecalciferol or calcium supplements in the studies by Orgel, Demirsoy or Claar et al, although the latter 2 studies did not clearly record methods of adverse event data collection. The study by Diaz et al was the only study to use calcitriol as the intervention of choice. This was reported to be well tolerated, with plasma and urinary calcium levels remaining within the normal range [275], although no numerical data or reference ranges were presented.

Table 19. Loke quality assessment for reporting of adverse events

Author, year	Study type	Any patients excluded from the adverse effects analysis	Which categories of adverse effects do the investigators report	How were these defined	Method of adverse event data collection	Did the report give numerical data by intervention group	Did the investigators report on all important or serious effects
Diaz, 2008 [275]	RCT	No	Hypercalcaemia Hypercalciuria	Not defined	Plasma and urine calcium measured every 3 months	Yes	No
Orgel, 2017 [276]	RCT	No	Hypervitaminosis Hypercalcaemia Nephrolithiasis Renal insufficiency Transaminitis	Not defined	Not reported	Yes	Yes
Demirsoy, 2017 [278]	Cohort	No	Hypercalcaemia Symptomatic nephrolithiasis	Not defined	Not reported	Yes	Yes
Young, 2018 [277]	Cohort	No	Supratherapeutic 25-OH D level Hypercalcaemia Nephrolithiasis	25-OHD>150ng/mL Communication with author: Hypercalcaemia (mg/dL): Age 8 weeks to 2 years:> 10.5, age 2-10 years:>10, age 10-18 years: >10.2, age 18-150 years:>10.1 Nephrolithiasis not defined	Retrospective chart review. Vitamin D levels measured every 3 months, calcium and vitamin D measured at each instance of nephrolithiasis	Yes	Yes
Claar, 2014 [279]	Cohort	Not reported	None specified	Not applicable	Not reported	No	No

2.3.2.12 Quality of evidence

Using the Grading or Recommendations, Assessment, Development and Evaluations (GRADE) approach to rating quality of evidence [280], (Table 20), there was moderate to low quality evidence that cholecalciferol supplementation at a dose of 100,000IU given 2 monthly for 3 doses has no impact on BMD, with low quality evidence that calcitriol supplementation also does not impact upon BMD. There was moderate quality evidence that cholecalciferol supplementation at a dose of 100,000IU 2 monthly for 3 doses increases levels of vitamin D in patients with ALL.

Table 20. Grading of recommendations, assessment, development and evaluations evidence profile: vitamin D for children with acute lymphoblastic leukaemia

Outcome of interest	Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias	Quality
Bone mineral density						
Orgel (RCT)	Nil serious	Nil serious	Nil serious	Nil serious	Undetected	High
Diaz (RCT)	Serious	Nil serious	Nil serious	Nil serious	Undetected	Moderate
Demirsoy (cohort)	Serious	Nil serious	Nil serious	Nil serious	Undetected	Very low
Calcium levels						
Orgel (RCT)	Nil serious	Nil serious	Nil serious	Nil serious	Undetected	High
Demirsoy (cohort)	Serious	Nil serious	Nil serious	Nil serious	Undetected	Very low
Vitamin D levels						
Orgel (RCT)	Nil serious	Nil serious	Nil serious	Nil serious	Undetected	High
Young (cohort)	Serious	Nil serious	Nil serious	Nil serious	Undetected	Very low
Demirsoy (cohort)	Serious	Nil serious	Nil serious	Nil serious	Undetected	Very low
Claar (cohort)	Serious	Nil serious	Nil serious	Serious	Undetected	Very low

2.3.2.13 Discussion

The purpose of this review was to evaluate the safety and efficacy of vitamin D in children and young people with ALL. Five studies were included for full analysis (2 randomised studies and 3 cohort studies), with a total of 191 included participants. The studies suggest that in the short term cholecalciferol supplementation does not impact upon BMD of patients with ALL, despite increasing vitamin D levels. Although one randomised study reported a greater LSBMD increment in the children with lower initial BMD in patients who received calcitriol, due to lack of clarity over the analysis, and despite biological plausibility, there were insufficient data to draw any conclusions from this [275].

Meta-analysis of study results was not possible due to marked statistical heterogeneity of reported outcomes and lack of studies with adequate sample sizes. Between the studies there was marked variability in patient populations, therapeutic intervention and duration of follow up, preventing pooling of results and subgroup analysis. All studies were limited by the short duration of follow up, which could reduce both detection of adverse events and any long-term benefits from treatment.

Several methodological approaches were used to assess risk of bias in the included studies. For RCTs the Cochrane risk of bias was used and study protocols were searched for. There was one high quality RCT with a published protocol, but with only 29 patients, conclusions drawn from this study must be limited [276]. There was a high risk of bias in the one other RCT, and their use of calcitriol, rather than cholecalciferol, together with lack of assessment of initial vitamin D status in patients, makes it difficult for the results of this study to be used in clinical practice [275]. In both of the RCTs supplementation to patients was only after induction chemotherapy was completed. The study by Orgel et al cited the *in vitro* results by Antony et al [281], as the reason for this delay. This study assessed proliferation of leukaemic cell lines in response to dexamethasone alone, and then dexamethasone with varying concentrations of calcitriol. However, there were a number of important limitations to this study, including lack of complete reporting of results, the *in vitro* nature of the study and lack of discussion of the relevance of the concentrations chosen in comparison with *in vivo* concentrations of therapeutic cholecalciferol. Therefore delaying treatment purely on the basis of these results may not be warranted.

For the cohort studies the ROBINS-I was used to assess risk of bias. The lack of consideration of potential confounders, including BMI, age, sex and

ethnicity limited the quality of results and led to serious risk of bias in all cohort studies included.

The lack of adherence monitoring in all but one of the papers may have limited the likelihood of significant findings. When adherence was assessed in one study, it found that less than 10% of prescribed doses were delivered [276]. This suggests that in studies with no assessment of adherence, the true adherence was likely to be limited, reducing the likelihood of any therapeutic benefit.

The Loke method for quality assessment of safety of vitamin D supplementation identified that the majority of studies were unclear on definitions and reporting methods of adverse event data collection. However, given these limitations, cholecalciferol appeared to be well tolerated, with no adverse events reported that would impact on patient safety.

2.3.2.14 Summary

This systematic review demonstrates that there is currently insufficient evidence to conclude that vitamin D supplementation in children and young people with ALL has any benefit on fracture incidence, BMD, incidence of osteonecrosis, survival time, quality of life, or response to ALL treatment. There were no studies which assessed use of vitamin D with respect to any of our patient centred primary outcomes. The studies that were included in this review did suggest that in young patients with ALL cholecalciferol has a good safety profile, although there were limitations with the adverse event reporting for the majority of studies.

Chapter 3 A retrospective national study of osteonecrosis in children and young people with acute lymphoblastic leukaemia

3.1 Introduction

Within haematology departments there has been an increasing awareness of ON as a significant consequence of ALL treatment, yet many aspects, including management and long term outcomes, remain unknown. For the UK population of young people being treated for ALL it is important that we accurately determine the prevalence and chronology of ON, identify risk factors and understand the management and long term consequences of the condition. This will enable clinicians to more accurately inform and prognosticate for patients who are at risk or who have developed ON during ALL treatment.

The primary aims of this study were to:

- Report the UK prevalence of symptomatic ON in young people with ALL recruited into UKALL 2003
- Describe the chronology of development of symptoms of ON and diagnosis
- Identify risk factors for the development of ON
- Determine joints affected by ON and methods of diagnosis of ON in patients with ALL
- Describe medical and surgical management of patients diagnosed with ON
- Establish long-term outcomes of patients affected by ON

This was undertaken in a cohort of patients enrolled to the national ALL trial for children and young adults, UKALL 2003, that ran from 2003 to 2011.

The methodology and results will be presented in this chapter, with a discussion of the results in Chapter 5.

3.2 Methods

3.2.1 Patient population

The patient population assessed in this study were the 3113 patients aged 1-24 years who were registered onto UKALL 2003 [128]. This was the

national ALL study which aimed to assess whether treatment intensity could be adjusted for children and young adults according to MRD risk stratification. Patients were recruited in 45 centres in the UK and Ireland between Oct 1, 2003 and June 30, 2011 and all patients had a diagnosis of ALL which was diagnosed with standard morphological and flow cytometric criteria [128, 282]. An outline of treatment regimens is presented in Figure 9. Initial risk stratification occurred at presentation, which was subsequently reviewed at day 15 and 28 of induction, with day 8 rather than day 15 used for patients on regimen B aged 1-15 year. At these points risk stratification incorporated cytogenetics and early response to induction therapy, assessed by bone marrow blast counts. Patients who started treatment on regimen A were aged < 10 years at diagnosis of ALL, with a highest WCC before starting treatment of less than $50 \times 10^9/L$. Regimen B was for patients defined at high risk of relapse because of their age (>10 years) or presenting white cell count ($\geq 50 \times 10^9/L$).

Patients were put onto regimen C if they either had a slow early response in regimen A or B, had MRD positive day 28 bone marrow and were randomised to regimen C, or had unfavourable cytogenetics (hypodiploidy, t(4;11), t(9;22), iAMP21, E2A-HLF) [128]. Patients with Philadelphia-chromosome-positive ALL were transferred onto other protocols once their Philadelphia chromosome status was known.

MRD low risk patients were randomly assigned to 1 (reduced treatment) or 2 (standard treatment) blocks of DI and MRD high risk patients were randomly assigned to standard treatment or regimen C, a more treatment intensive schedule. Patients with indeterminate MRD status at day 28 received 2 blocks of DI. An outline of risk groups and randomisations is presented in figure 10.

In 2009 the randomisation of MRD low risk patients to 1 or 2 blocks of DI was closed due to accrual of the target number of patients, and subsequent patients received a block of single delayed intensification.

Dexamethasone doses:

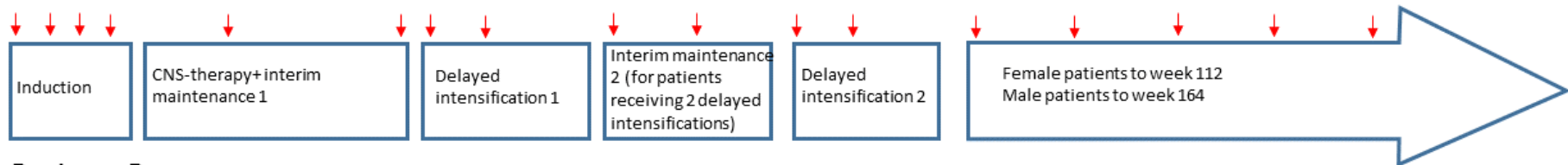
All patients received a daily dose of $6 \text{mg}/\text{m}^2/\text{day}$ oral dexamethasone during induction for 28 days with a maximum dose of 10mg. Patients on regimen A and B received dexamethasone during interim maintenance, at a daily dose of $6 \text{mg}/\text{m}^2$ oral dexamethasone on days 1-5 and days 29-33 in regimen A, or days 2-6 and days 30-34 in regimen B. In those who received 2 blocks of DI, they also had this on week 24 and 28 in interim maintenance 2. In DI, all

patients received 10mg/m² dexamethasone daily for two weeks, on alternate weeks, with no cap on the dose. Maintenance therapy was run in 12 week cycles, and in each cycle patients were given 6mg/m²/day oral dexamethasone on days 1-5, 29-33 and 57-61 of each cycle.

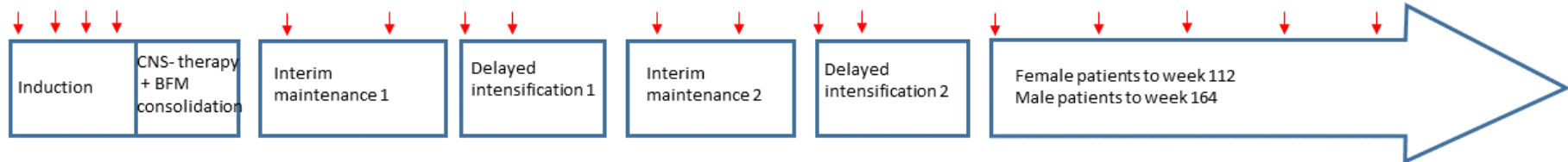
In UKALL 2003 the upper age limit of entry was 18 years at the start of the trial, but increased to 20 years in February 2006, and to 24 years from August 2007. This was due to retrospective studies showing improved outcomes in young adults when treated on paediatric protocols. In June 2008 the overall treatment intensity for patients with Down syndrome was reduced due to excess treatment-related mortality. From June 2008 Down syndrome patients were registered on the trial but did not undergo randomisation and were treated as clinical standard-risk patients, with adjustment of post-induction treatment according to response to induction therapy [128].

Figure 9. Outline of UKALL 2003 treatment regimens

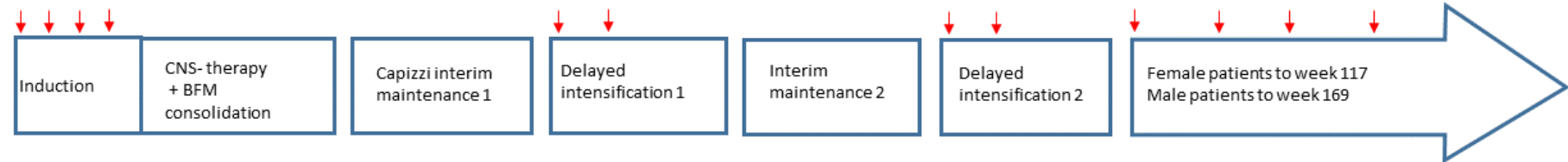
Regimen A:



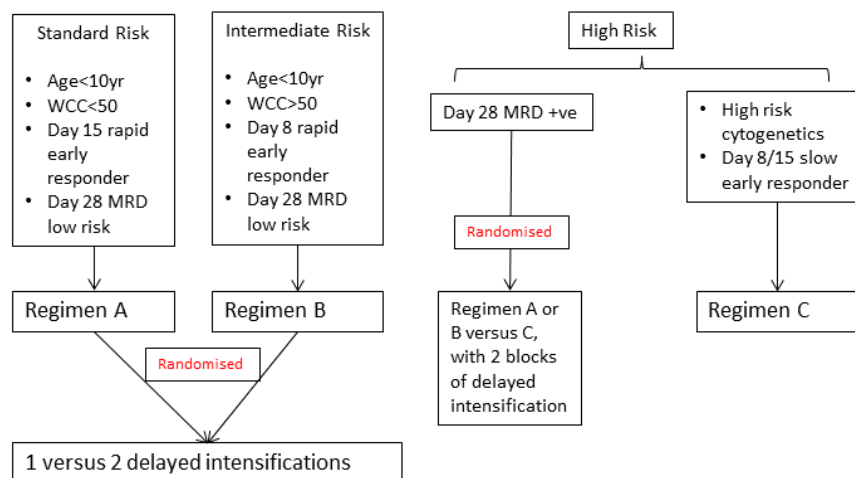
Regimen B :



Regimen C :



↓ Represents 5-7 days of dexamethasone (see text for full details).

Figure 10. Treatment and randomisation algorithm in UKALL 2003

3.2.2 Questionnaire development

In UKALL 2003 long-term monitoring of patients who developed ON was not a routine part of data collection, although there was reporting of patients with severe ON. I therefore developed a questionnaire in collaboration with the Consultant Paediatric Haematologists at Leeds Children's Hospital to understand more about the prevalence, management and outcomes of patients with ALL who developed symptomatic ON (Appendix 3). A questionnaire was felt to be the most appropriate method of data collection as it is a practical method of data collection from a large cohort of people. It was important to carefully consider the design of the questionnaire, to ensure readability, ease of use, and ease of analysis. Key areas for data collection were identified from the literature review (Chapter 2.1), with a focus on areas of insufficient or inconclusive data, such as time to diagnosis, medical and surgical management and long term outcomes. It was important to include questions on the chronology of the development of ON, including when symptoms were first reported, and timing of diagnosis. The aim of this was to establish if there were significant delays in diagnosis of ON in this population. Method of diagnosis and reports of all diagnostic imaging were requested, to enable us to verify the diagnosis. The feasibility of requesting imaging directly was considered. This would allow grading and true confirmation of ON, but at the time of initial data collection it was felt that there was not sufficient technical expertise within the team for this to be appropriately utilised.

There is limited information about the medical and surgical management of patients. This was an important area I wished to explore to allow an

understanding of patient care across the country. Specific information regarding cessation of steroids, use of bisphosphonates, vitamin D and surgical interventions was requested. These were chosen as all have been previously been considered as potential therapeutic interventions in the management of ON, although as previously discussed, evidence is limited.

Throughout the questionnaire closed questions were used when possible, providing the respondent with a rapid method of indicating their response, and increasing ease of analysis. There were areas where open questions were felt to be more appropriate, including symptomology and types of surgery. This was to prevent 'suggestion' of answers for symptoms felt to be attributed to ON, and to allow data collection on the full range of surgical procedures undertaken.

Prior to distribution of the questionnaire the data manager at Leeds Children's Hospital, together with other data managers working in the UK, assessed readability and clarity of the questionnaire. This helped to ensure that questions were as objective as possible, with minimal ambiguity. I subsequently piloted this on 10 patients in Leeds Children's Hospital. Our data manager also tested the questionnaire separately on a sample of these 10 patients. Success of the pilot was assessed by appraising ease of data collection and questionnaire use, time taken to complete the questionnaire and readability of the questionnaire. This included consideration of factors such as layout of the questionnaire, and avoiding bias in questions. It was important that the questionnaire was as clear, concise and as direct as possible, with avoidance of redundant questions. After piloting the questionnaire, minor changes were made to layout and wording of questions, prior to national distribution.

In the final questionnaire, areas of data collection included:

- Patient demographics
- Joints affected by ON
- Timing of onset of symptoms
- Timing of diagnosis of ON
- Fracture history
- Method of diagnosis of ON
- Medical management of ON
 - cessation of steroids
 - bisphosphonate use
 - vitamin D supplementation
- Surgical management of ON

- Long term effects:
 - no long term effects
 - minimal disability (able to carry out activities of daily living)
 - significant disability (unable to carry out activities of daily living)
 - requires a wheelchair
 - death (any cause)

3.2.3 Data Source

The central trial unit (Clinical Trials Service Unit) for UKALL 2003 was notified of patients who developed bone toxicity (including ON, osteopenia and fractures) during their leukaemia treatment. Reporting was via toxicity reporting forms (Appendix 5), which specifically requested data regarding ON, or serious adverse event forms. A serious adverse event was defined as any adverse event that was life-threatening, required unexpected hospitalisation or prolongation of a hospital stay, resulted in persistent or significant disability or incapacity, or resulted in death.

After receiving approval from the Chief Investigator of UKALL 2003 a list of these patients from the Clinical Trial Service Unit was obtained, together with demographic and treatment details, including age at diagnosis of ALL and ethnicity data. Ethnicity was determined by self-report, and categorised into the following groups: Black, White, Asian, Oriental, Middle Eastern, Mediterranean, mixed, other, unknown.

3.2.4 Questionnaire distribution

Between 08/04/2015 and 20/04/2015 each of the 40 primary treatment centres (PTCs) were contacted via NHSmail, a secure e-mail service authorised for sending sensitive information. Contact was made with the data manager, research nurse and consultants caring for these patients, listing patients identified by the central trial unit who were reported to have suffered from bone toxicity in their centre, based on information from toxicity reporting forms. The questionnaire could be completed by any of these people. A questionnaire was attached to the e-mail for completion for each patient, together with a supplementary form developed to identify and gain further information on those patients who were not previously reported to have bone toxicity (Appendix 4).

3.2.5 Managing returns and data collection

Up to 5 reminders were sent out to centres over the course of 8 months to improve questionnaire uptake. All completed questionnaires were sent back to myself, together with imaging reports.

3.2.6 Statistical analysis

An Access database was developed in order to store and collate questionnaire data. Access was chosen as the most appropriate format for this as it readily allows data cleaning, data validation and development of data searches and reports. Seven tables were constructed to allow thematic collation of data, based on the main sections of the questionnaire. Free text responses were coded to allow ease of data analysis. Once data entry was complete, queries were generated to allow further analysis. Height and weight variables had to be dropped from analysis as data were missing for over 80% of patients.

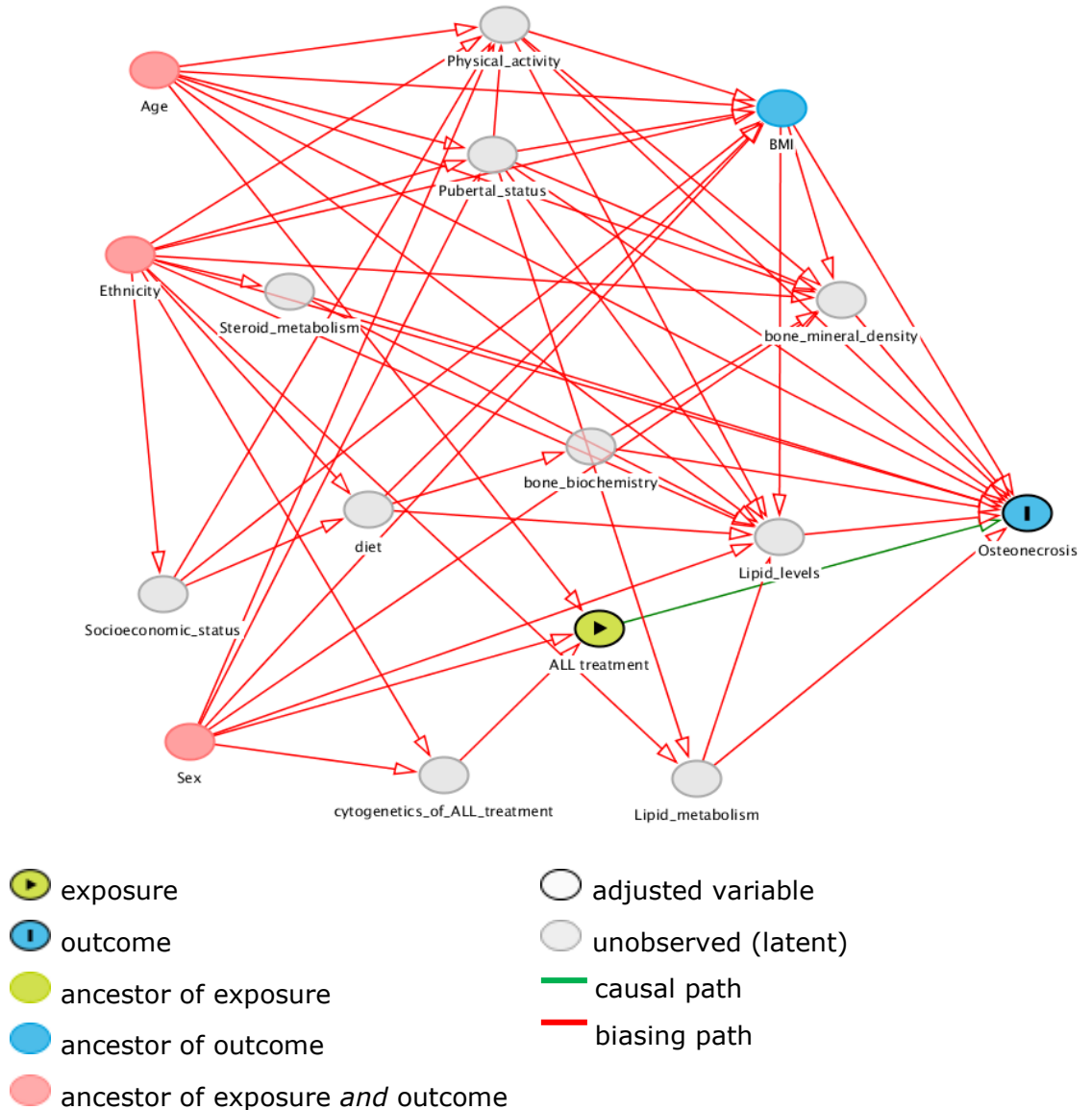
Descriptive analysis, including medians, cumulative incidence, and interquartile range (IQR) was used to describe the prevalence of ON in the cohort of patients and chronology of development of symptoms of ON and time to diagnosis. Percentages were used to describe the joints affected by ON, the management of ON and long term outcome of patients affected by ON. Sub-analysis was undertaken for those patients who received bisphosphonates and those who had surgical interventions for ON.

Univariable and multivariable logistic regression analyses were used to identify significant differences in the prevalence of ON according to age group at diagnosis (age < 10, 10-15 and 16+ years), sex, ethnicity (White, Black, Asian, other), and treatment (1 or 2 rounds of DI). Ethnic groups other than Black, White and Asian were combined due to the small numbers of patients in these groups. Odds ratios and 95% confidence intervals were reported as measures of association.

The minimal sufficient set of confounders adjusted for was based on a causal inference framework identified by the drawing of a DAG [283, 284] (Figure 11, model code in Appendix 7). A DAG is a graphic model depicting a set of hypotheses about the causal process, which in turn generates a set of variables of interest. The use of DAGs is a mathematically rigorous method for minimising bias and determining true confounders [284]. The causal diagram is developed as a graphic model by explicitly defining the theoretical causal relations between each covariate (including the main exposure; in this case, ALL treatment) and outcome (ON). Single-headed

arrows represent assumed direct links from cause to effect. Causal paths start at the exposure, contain only arrows pointing away from the exposure, and end at the outcome. Biasing paths are all other paths from exposure to outcome. Emphasis is placed on assuming a theoretical relationship exists between every pair of covariates unless there is convincing evidence of a null relationship. Each covariate in turn can be considered as the main exposure variable so that bespoke model adjustment can be made thus avoiding the table 2 fallacy [285], whereby multiple adjusted effect estimates from a single model are presented in a single table.

Figure 11. Directed acyclic graph for causal effect identification in development of osteonecrosis



Using the DAG from Figure 11, the results suggested that the minimal sufficient adjustment sets for estimating the effect of number of DI blocks on development of ON were age, sex and ethnicity. No adjustment was necessary to estimate the total effect of ethnicity, age or sex on development of ON. The model suggests that the total effect of treatment regimen cannot be estimated by covariate adjustment, hence further analysis of this was not undertaken.

Multivariable logistic regression was therefore only performed for assessment of the impact of number of DI blocks, which was adjusted for age, sex, and ethnicity, with univariable logistic regression used for age, sex and ethnicity.

Statistical analysis was undertaken using Stata version 14 (StataCorp, 2015) (Appendix 6 for Stata code). The directed acyclic graph (DAG) was developed and analysed using DAGitty version 2.3, a web-based software program for analysing causal diagrams.

3.2.7 Ethical permission

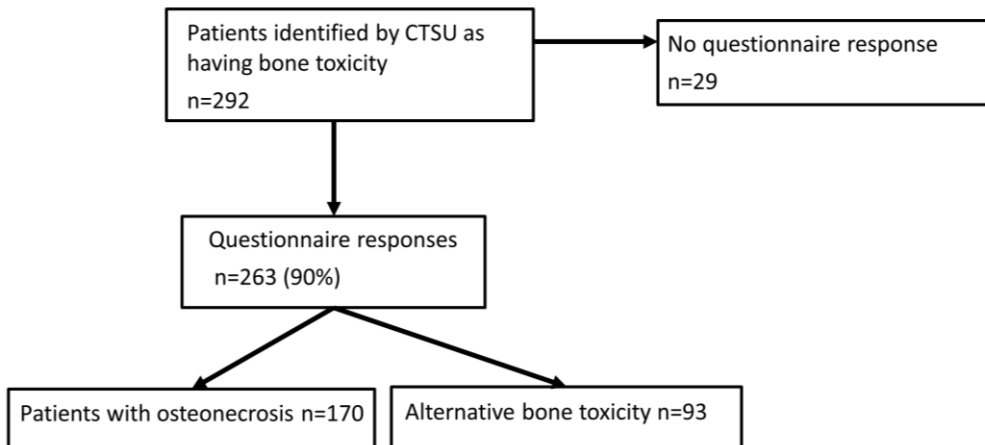
Consent for this work was covered by existing consent for UKALL 2003, ISRCTN07355119.

3.3 Results

A total of 292 patients were reported by the Clinical Trials Service Unit as having some form of bone toxicity (which included symptomatic severe osteopenia, fracture and avascular necrosis) and questionnaires were completed for 90% of these patients. There was no explanation given for the lack of questionnaire completion in 25 of the 28 cases where there was no response (23 centres), and notes were not available for the remaining 3 patients (Appendix 8 provides details of questionnaire responses according to centre). The median duration of follow-up for patients from time of ALL diagnosis was 70.5 months (range 24-127 months, IQR 54-86 months).

3.3.1 Prevalence of osteonecrosis in UKALL 2003

In the cohort of 264 patients for which results were obtained, 170 patients were reported to have ON (Figure 12). This is 55 more patients than were formally reported via toxicity reporting alone [128]. Given the total number of patients recruited to UKALL2003 was 3113, the overall prevalence of ON in the cohort of patients recruited to UKALL 2003 was thus calculated to be 5.5%.

Figure 12. Questionnaire response rate for patients with bone toxicity

Demographic details of the 29 patients with no questionnaire response, compared with all patients enrolled into UKALL 2003 and patients with radiographically confirmed ON, are provided in Table 21. Patients for whom no questionnaire response was received were not included in the overall analysis of patients, as ON was not able to be confirmed. The p-value (calculated using a Chi-squared) represents the comparison between columns 2 and 3. Although there were significantly more patients aged over 10 years for whom questionnaire responses were not available, the absolute number of patients was small (n=26). There were no other areas in which the patients with no questionnaire responses differed significantly from those for whom we were able to confirm a diagnosis of ON.

Table 21. Demographic details of patients

Demographic	Number of patients with radiographically confirmed osteonecrosis (n=170) (%)	Number of patients with no questionnaire response (n=29) (%)	P-value	All trial patients (n=3113) (%)
Age (years) at diagnosis of ALL				
<10	22 (13%)	3 (10%)	0.65	2279 (73%)
10-15	111 (65%)	10 (34%)	0.002	607 (19%)
16+	35 (21)	16 (55%)	<0.001	227 (7%)
Ethnicity				
White	141 (83%)	25 (86%)	0.69	2525 (81%)
Asian	15 (9%)	2 (7%)	0.72	74 (2%)
Black	3 (2%)	1 (3%)	0.73	232 (7%)
Other	11 (6%)	1 (3%)	0.52	164 (5%)
Unknown/missing	0 (0%)	0 (0%)	n/a	118 (4%)
Gender				
Male	96 (56%)	19 (66)	0.32	1767 (57%)
Female	74 (44%)	10 (34)	0.32	1346 (43%)

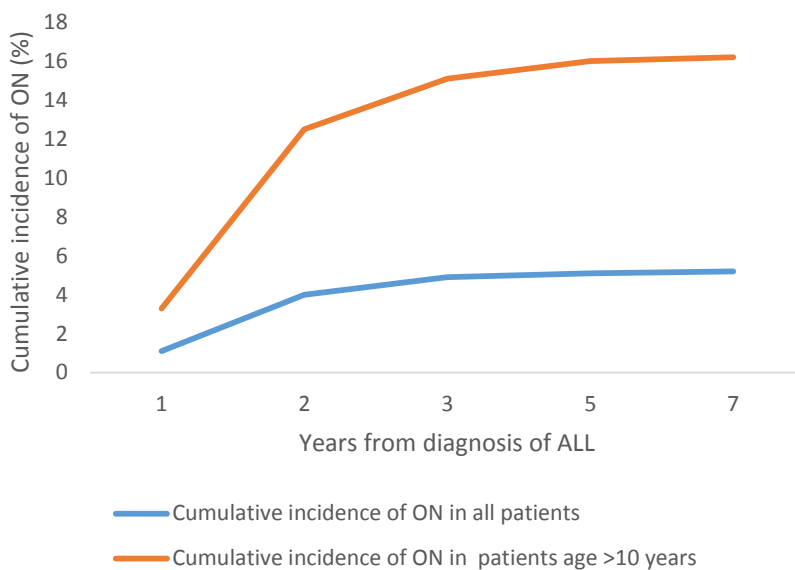
3.3.2 Chronology of symptomatology and diagnosis of osteonecrosis in patients with acute lymphoblastic leukaemia

In this cohort of patients, symptoms of ON were reported in 139 patients, and were typically pain and/ or reduced range of movement. Symptoms of ON presented in lower limbs for 118 patients (85%), both upper and lower limbs for 6 patients (4%) and upper limbs for 12 patients (9%). Back pain was the presenting symptom of ON in 2 patients (1%).

Symptoms of ON were reported at a median of 14 months after diagnosis of ALL (IQR 10-19 months). The date of diagnosis of ON was not available for 6 patients. Of the remaining 164 patients, ON was diagnosed at a median time of 16 months after diagnosis of ALL (IQR 12-22 months).

In patients who presented with upper limb symptoms, the median time from diagnosis of ALL to development of symptoms of ON was 17 months (range 9-32 months, IQR 14-21 months), compared with 13.5 months for those who presented with lower limb symptoms (range 1-72 months, IQR 10-19 months), but there was not found to be a significant difference between the groups ($p=0.60$, CI -8.58 to 5.00).

In the 1st year after diagnosis of malignancy, 35 patients were diagnosed with ON (21% of all patients diagnosed with ON), with 91 patients diagnosed during the 2nd year (55%), and 25 diagnosed during the 3rd year (15%). Eight patients were diagnosed between 3 and 5 years after diagnosis of malignancy (5%), and only 2 patients were diagnosed with ON after 5 years (1%). The longest time to diagnosis of ON was 6.26 years after diagnosis of ALL. The cumulative incidence of ON diagnosed in all patients was 1.1% at 1 year, 4.0% at 2 years, 4.9% after 3 years, 5.1% at 5 years and 5.2% at 7 years. For patients over the age of 10 at diagnosis of ALL, the cumulative incidence of ON was 3.3% at 1 year, 12.5% at 2 years, 15.1% at 3 years, 16% at 5 years and 16.2% at 7 years (Figure 13).

Figure 13. Cumulative incidence of osteonecrosis in UKALL 2003

3.3.3 Risk factors associated with development of osteonecrosis

Age, ethnicity, sex and 1 versus 2 blocks of DI were assessed as risk factors in development of ON. Univariable and multivariable analysis was undertaken using the results of the DAG assessment described previously (Tables 22 and 23).

Table 22. Results of univariable logistic regression analysis of age, sex and ethnicity, and association with osteonecrosis in patients with acute lymphoblastic leukaemia

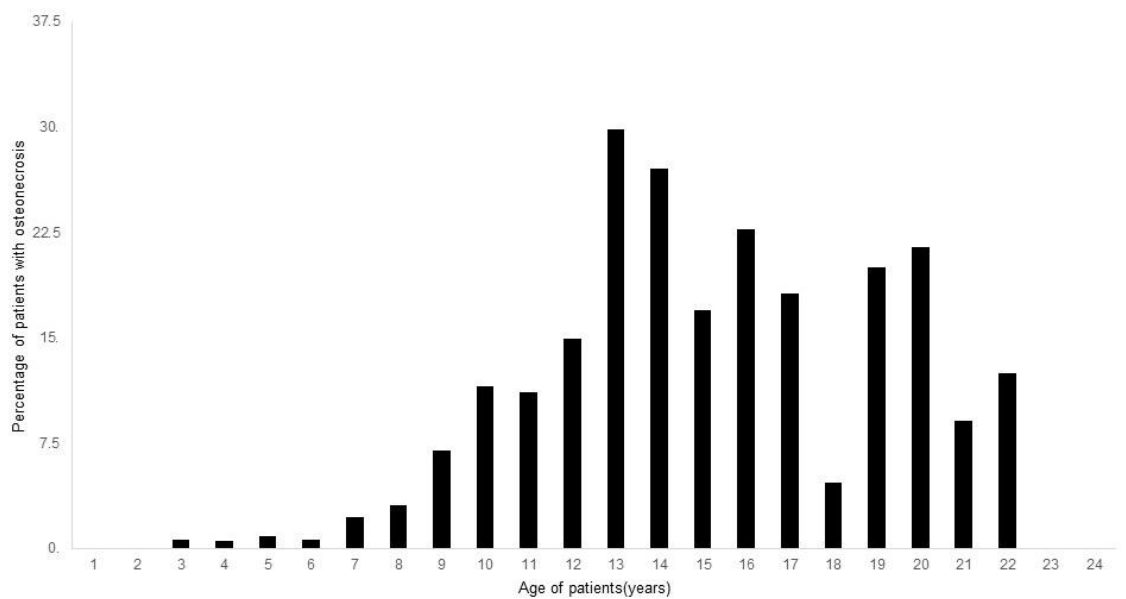
				Univariable logistic regression		
Variable		With osteonecrosis (frequency (%))	Without osteonecrosis (frequency (%))	Odds ratio	95% Confidence intervals	P-value
Age (years)						
	<10	22 (1)	2257 (99)			
	10-15	111 (18)	496 (82)	22.96	14.38-36.64	<0.001
	16-20	32 (17)	154 (83)	21.31	12.09-37.57	<0.001
	21+	3 (7)	38 (93)	8.10	2.32-28.22	0.001
Ethnicity						
	White	141 (6)	2384 (94)			
	Black	3 (4)	71 (96)	0.73	0.23-2.35	0.60
	Asian	15 (6)	217 (94)	1.20	0.69-2.07	0.05
	Other	11 (4)	271 (96)	0.91	0.52-1.59	0.73
Gender						
	Male	96 (5)	1671 (95)			
	Female	74(5)	1272 (95)	1.04	0.76-1.43	0.79
No. of blocks of delayed intensifications						
	2	138 (6)	2142 (94)			
	1	31 (4)	802 (96)	0.85	0.59-1.22	0.38

Table 23. Results of multivariable logistic regression analysis of number of blocks of delayed intensification and association with osteonecrosis in patients with acute lymphoblastic leukaemia

				Univariable logistic regression			Multivariable logistic regression		
Variable		With osteonecrosis (Frequency (%))	Without osteonecrosis (Frequency (%))	Odds ratio	95% Confidence intervals	p-value	Odds ratio	95% Confidence intervals	p- value
No. of delayed intensifications									
	2	138 (6)	2142 (94)				1.00		
	1	31 (4)	802 (96)	0.85	0.59-1.22	0.38	0.99	0.67-1.45	0.94

It is clear that age at diagnosis of ALL was an important risk factor for the development of ON, with the highest risk of developing ON in the 10-20 year age group. Patients over 20 years of age at diagnosis of ALL were still at greater risk of developing ON compared with those under 10 years of age, but at a lower risk than those aged between 10 and 20 years at diagnosis. The full distribution of age of patients who developed ON is illustrated by Figure 14, with the x-axis representing the age at which ALL was diagnosed [286].

Figure 14. Age of patients in UKALL 2003 with symptomatic osteonecrosis.



A contingency table was used to assess if there was any age by gender interaction for development of ON. The results in Table 24 suggest the lack of significance, which was confirmed in a likelihood ratio test, where the addition of age by gender interaction for categorical variables showed no significance ($p=0.96$).

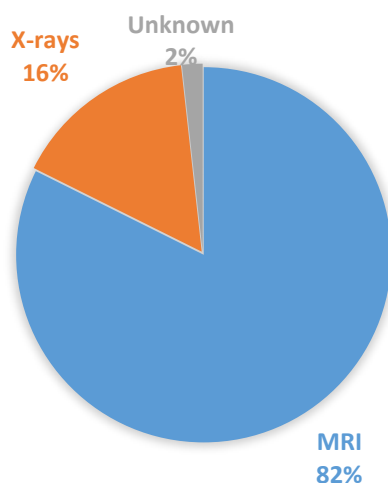
Table 24. Age by gender interaction for development of osteonecrosis

Age	Females		Males	
	Without ON (%)	With ON (%)	Without ON (%)	With ON (%)
<10	1005 (98.82)	12 (1.18)	1252 (99.21)	10 (0.79)
10-15	207 (81.18)	48 (18.82)	289 (82.10)	63 (17.90)
16-20	51 (83.61)	10 (16.39)	103 (82.40)	22 (17.60)
21+	12 (92.31)	1 (7.69)	26 (92.86)	2 (7.14)

3.3.4 Method of diagnosing osteonecrosis

MRI was the most common method of imaging to confirm the diagnosis of ON in UKALL 2003, used in 140 (82%) patients. Plain X-rays were used for diagnosing ON in 27 patients (16%) across 10 centres. There appeared to be centre specific variation in method used to diagnose ON, with two large centres diagnosing around 40% of cases of ON using X-ray. The diagnostic imaging modality was unknown in 3 patients (Figure 15). Of those that had X-rays to diagnose ON, 16 patients subsequently had MR imaging (59%). All of the imaging reports were reviewed to verify the diagnosis, with no inaccurate diagnoses made.

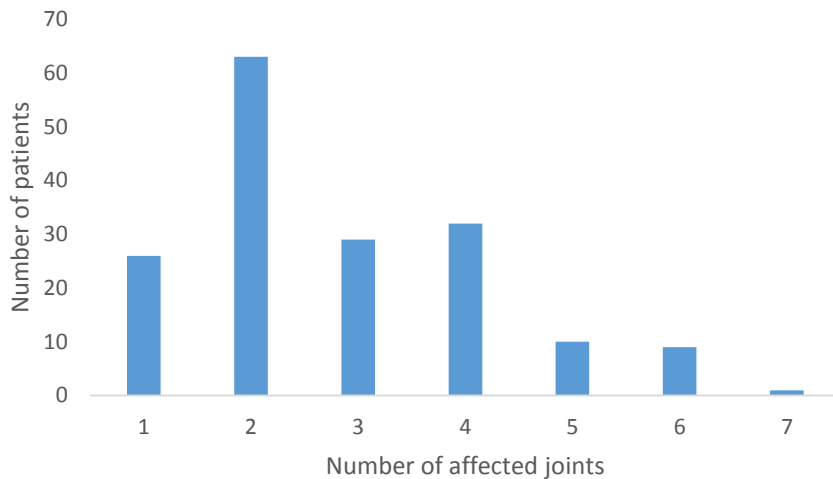
Figure 15. Imaging modality used for diagnosis of osteonecrosis



3.3.5 Joints affected by osteonecrosis

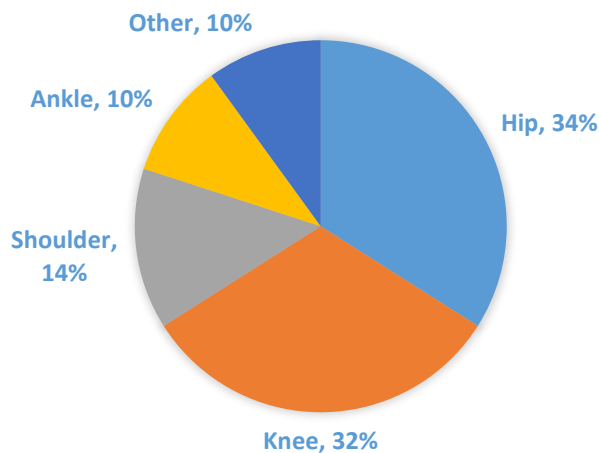
Of the 170 patients who developed ON, there were a total of 480 joints with confirmed ON. Fifteen percent of patients (n=26) had unifocal ON. In those under the age of 10 years at diagnosis of ALL, of those who developed ON (n=22), 5 patients had unifocal ON (23%).

Figure 16 illustrates the distribution of number of joints affected in patients with osteonecrosis.

Figure 16. Number of joints affected in patients with osteonecrosis

In the 170 patients, hips (34%, n=165), knees (32%, n=154), shoulders (14%, n=67) and ankles (10%, n=46) were the most commonly affected joints (Figure 17). A total of 99 patients (58%) had at least 1 hip affected, and of these 59 people (35%) had 2 affected hips.

A total of 41 (24%) people had at least one shoulder affected by ON, with 35 (21%) of these having both shoulders affected. There were 87 people (51%) who had at least one knee affected by ON, of which 76 patients (45%) had both knees affected. Of the 99 patients who had at least one hip affected by ON, 32 patients (32%) also had at least one knee affected, and 29 had at least one shoulder affected (29%). Only 18 patients (11%) with ON had no hips or knees affected.

Figure 17. Joints affected by osteonecrosis

3.3.6 Medical management of patients

There was significant variation in the medical management of patients across different PTCs in the UK.

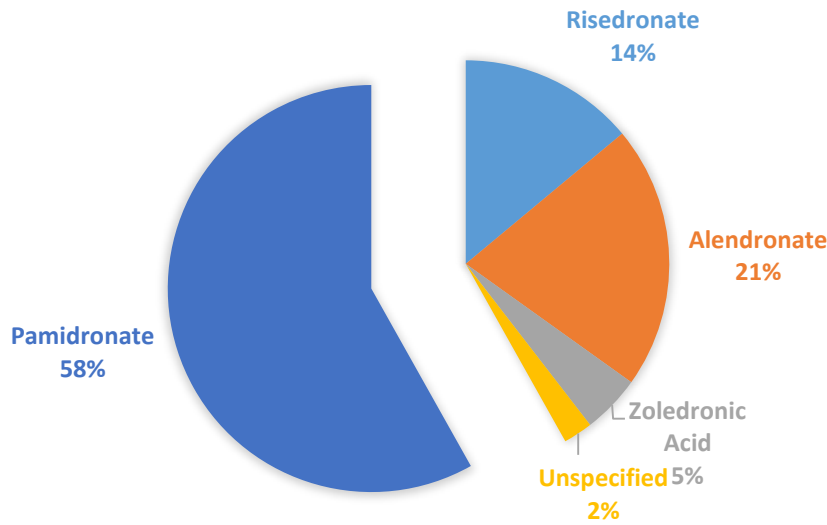
3.3.6.1 Steroid management

In the UKALL 2003 protocol there was initially no specific guidance about continuation or cessation of steroids after a diagnosis of ON. From 2009, centres were advised to contact the Trial Coordinators if ON developed before maintenance therapy, and for further steroids to be omitted if ON developed during maintenance. In 60% of cases steroids were stopped (although the specific timing of stopping steroids was not collected). In 32% of cases steroids were continued, and in 8% of cases it was unknown whether steroids were stopped due to ON. There were 3 centres in which all the patients who developed ON had their steroids stopped (Addenbrooke's, Sheffield, North Staffordshire), others where the vast majority of patients had steroids stopped (Bristol, Manchester, Southampton), and others where the majority of patients continued on steroids after diagnosis of ON (Our Lady's Children's Hospital in Ireland, Leeds Children's Hospital). The date and rationale for cessation of steroids was not requested. In 3 of the patients it was reported that dexamethasone was changed to prednisolone subsequent to the diagnosis of ON.

3.3.6.2 Use of bisphosphonates

Bisphosphonates were used in 27% of patients with ON (n=43). In the majority of centres intravenous pamidronate was used as the bisphosphonate of choice, although in one centre (Christie Hospital, Manchester, which is a teenage/ adult institution) all patients received oral alendronate. In Birmingham the 2 patients that were given bisphosphonates were given zoledronate, and in Manchester Children's Hospital of the 9 patients that were given bisphosphonates, 6 were given oral risedronate. Figure 18 illustrates frequency of different forms of bisphosphonate used across all centres. Of the 40 centres involved in this study, 13 used bisphosphonates in some of their patients with ON. Information regarding indication, duration and dose of bisphosphonate was not collected.

Figure 18. Type of bisphosphonates given to patients with osteonecrosis



In the 170 patients reported to have ON, 35 were reported to have fractures (21%). Of the 43 patients who were given bisphosphonates, 12 patients were reported to have fractures (28%), and 13 were reported to have had an assessment of their BMD. Of these 13 patients we were able to obtain the initial DXA reports for 9 patients, and 7 out of 9 patients had a lumbar spine Z score of <-2 SDS.

3.3.6.3 Vitamin D use in patients with osteonecrosis

Vitamin D supplementation was provided to patients in 32% of cases. 36% of patients received no supplementation, whilst provision was unclear in the remaining 32% of cases. Details regarding vitamin D sufficiency, treatment modality or date of initiation of treatment were not requested.

3.3.7 Surgery in patients with osteonecrosis

Of the 170 patients who were diagnosed with ON, 65 were reported to have had surgery related to their ON (38%). These 65 patients underwent a total of 99 surgical procedures, with a number of centres reporting that patients were awaiting further surgical events [286]. The surgical procedures performed are shown in Table 25.

Table 25. Surgical procedures reported in patients with osteonecrosis

Type of surgery	Number of patients (%)
Hip replacement	33 (19)
Core decompression	22 (13)
Knee replacement	2 (1)
Shoulder replacement	2 (1)
Arthroscopy	10 (6)
Hip fixation	2 (1)
Other	10 (6)

Total hip replacements (THR) were the most common surgical procedure described in our cohort of patients. Core decompression, which involves drilling into the area of ON, was the second most common surgical procedure reported. In the 10 patients who had arthroscopy, only 3 received arthroscopy alone. Additional procedures undertaken included synovial debridement, meniscotomy, correction of osteochondral defects, reshaping of the femoral head, removal of loose bodies and joint stabilisation. Multiple joint replacements were required in 16 patients, with 12 patients requiring bilateral THR. The 2 patients who needed shoulder replacements also needed a hip to be replaced, and 1 patient needed a knee and hip to be replaced. There was 1 patient who had 3 joints replaced (bilateral THR and one knee replacement). The median age at which joint replacement was performed was 19.25 years. The youngest age at which a joint was replaced was 12.9 years.

Of the 22 patients who were under 10 years of age at diagnosis of ALL and who developed ON, only 4 (18%) had any form of surgical management (2 had core decompression, 2 had joint replacements). The youngest age of joint replacement was 8 years.

When affected hips, knees, ankles and shoulders were assessed in turn, the following results were found:

3.3.7.1 Hips

Of the 165 hips affected by ON, there were 47 THRs reported (28% of all hips affected), with 22 core decompressions described (13%).

Other procedures identified were:

- Hip fixation: n=4
- Reshaping of femoral head: n=2
- Removal of femoral spurs: n=2
- Osteoplasty: n=1
- Osteotomy: n=1
- Articulated distraction: n=1

3.3.7.2 Knees

A total number of 154 knees were reported to be affected by ON. Of these, 3 had knee replacement (2%), and 5 (3%) had core decompression. There were arthroscopies performed in 5 knees (3%) affected by ON.

3.3.7.3 Shoulders

There were 67 shoulders affected by ON, and of these, 3 (4%) went on to be replaced, and 2 core decompressions were performed (3%).

3.3.7.4 Ankles

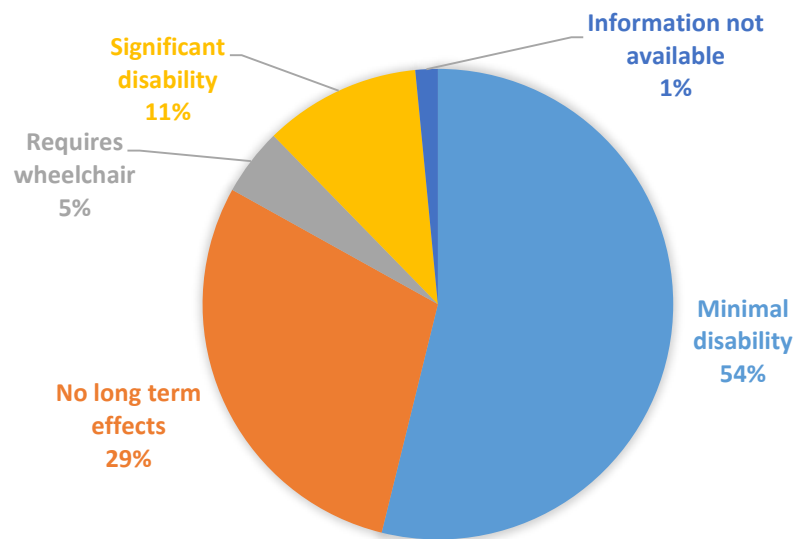
Of the 46 ankles reported to be affected by ON, 3 (6.5%) had core decompression performed.

3.3.8 Long-term outcomes of patients affected by osteonecrosis

In the questionnaire distributed to centres, the long term effects of ON were defined as the effect of ON at the most recent follow-up consultation.

Despite the high incidence of surgery in patients affected by ON, at the time of data collection (median 70.5 months follow-up) the majority of patients who had ON were reported to have either no long term effects (39%, n=66), or minimal disability (38%, n=64). Significant disability was reported in 9% of patients (n=16), and 5 patients required a wheelchair (3%). Six percent of patients had died at time of data collection, and information was not available for 9 patients (5%). These results are illustrated in Figure 19. This distribution was similar for patients both under and over 10 years of age at diagnosis of ALL.

Figure 19. Long term outcomes of patients affected by osteonecrosis who had surgical intervention



Of the patients who had surgery, 54% (n=35) were reported to have minimal disability and 29% reported no long term effects. Despite surgical intervention 7 patients (11%) still described the presence of significant disability. 3 patients who had surgical intervention (arthroplasty in 2 patients, core decompression in 1 patient) required a wheelchair at the time of data collection.

3.4 Detailed analysis of surgical and radiographic outcomes in children and young adults in UKALL 2003 affected by osteonecrosis

3.4.1 Introduction

The previously described retrospective national study of ON in patients recruited into UKALL 2003 highlighted the considerable surgical burden of ON [286]. However, in the initial data collection the high prevalence of surgery was unanticipated, and detailed information was not requested.

The primary aim of this additional work was to gain further information about surgical procedures used in different joints affected by ON, ascertain the timing of procedures and the use of sequential procedures in the management of this challenging condition. These results are described in part A, whilst part B uses radiographic staging of osteonecrotic lesions to assess the effectiveness of core decompression in preventing joint collapse for patients with ON affecting the femoral head.

3.4.2 Methods

The methodology for identifying patients with ON has previously been described in Chapter 3.2.

A further letter of contact and a questionnaire was developed in collaboration with the paediatric orthopaedic team and the paediatric haematology team (Appendix 9). Prior to distribution the data manager at Leeds Children's Hospital assessed readability and feasibility of returning the information requested.

All centres who had previously reported patients with ON in UKALL2003 were contacted again with a short questionnaire for completion. The following information was requested for each reported patient:

- Operation notes
- Orthopaedic clinic letters
- Radiographic imaging of areas of ON
- Current levels of pain
- Current mobility status

3.4.2.1 Questionnaire distribution

Between February 2018 and July 2018 each of the PTCs were contacted via NHSmail. Data managers and research nurses were contacted in each

centre, and patients were listed according to their trial number and date of birth.

Up to 3 reminders were sent out to centres over the course of the 6 months to improve questionnaire uptake.

3.4.2.2 Analysis

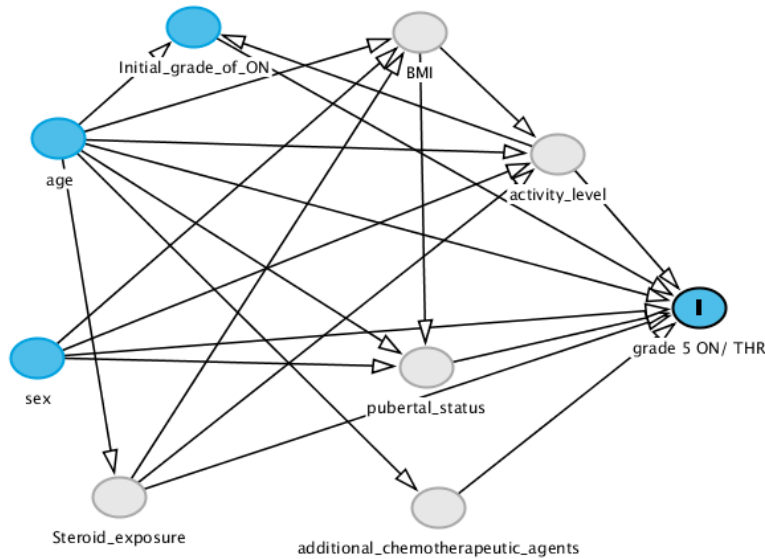
The initial Access database developed for collection of UKALL2003 retrospective data was modified to allow input of additional clinical data from this work, allowing integration of previously collated data. Pain and mobility status were coded to allow ease of data analysis. Pain was categorised into no pain/ occasional pain relief required/ regular pain relief required. Mobility status was categorised into the following groups: requiring wheelchair; requiring crutches; limited mobility; full mobility. Where available, details from operation notes were extracted and inputted into the central database.

For Part A, descriptive analysis, including medians, percentages, chi-squared test and IQR was used to describe the demographics of the population, timing of surgical interventions and surgical procedures used.

For Part B, radiographs were scored by a tertiary paediatric radiologist as per the scoring system developed by Niinimäki et al in order to determine the stage and extent of the osteonecrotic lesion [167]. The diagnostic MRI was used to grade the lesion. Subsequent imaging was used to determine final grade of ON. Plain radiographs were only used to determine the presence or absence of collapse of the femoral head (grade 5 ON).

A Kaplan-Meier failure time plot was used to estimate and graphically summarise time from initial diagnosis of ON to the end-point (grade 5 ON (joint collapse)/ total hip replacement (THR)). When the end-point was not reached the patient was censored. The Cox proportional hazards model was used to compare the use of core decompression with no joint preserving surgical intervention, with Breslow's method for ties to adjust for the natural clustering of joints within patients [287]. The patients with grade 4 ON at diagnosis were analysed as a subset in an ad hoc subgroup analysis. A causal inference approach using DAGs was used to determine the need for covariate adjustment (Figure 20), showing that no additional adjustment was necessary to estimate the total effects for age or sex (DAG code available in Appendix 7). The total effect for initial grade of ON could not be estimated by covariate adjustment. Statistical analysis was undertaken using Stata version 14 (StataCorp, 2015) and dagitty software was used for development of the DAG [283].

Figure 20. Directed acyclic graph for causal effect identification of development of grade 5 osteonecrosis



- outcome
- ancestor of outcome
- unobserved (latent)

3.4.3 Results: Part A

Surgical management of patients with osteonecrosis

Of the 170 patients reported to have developed ON during UKALL2003, further information was received for 85 patients (50%) from 14 centres. The main reason for lack of supply of further information was insufficient data manager capacity, or lack of capacity to supply radiological images on disk. Median duration of follow up was 83 months for these 85 patients.

Sixty surgical operation notes were available for review (64%), and additional details regarding the surgery are provided for these patients.

3.4.3.1 Demographics

Demographic details of patients for whom information was received, compared with all patients with confirmed ON are provided in Table 26. It can be seen that there are no significant differences between the two groups.

Of the 85 patients with follow-up data, the median age of patients at diagnosis of ALL was 13.83 years (IQR: 11.79-15.54 years), with a median age at diagnosis of ON of 15.21 years (IQR: 13.17-17.04 years).

Table 26. Comparison of demographic details for all patients with confirmed osteonecrosis with those for whom secondary questionnaire data was received

Demographic	Patients with confirmed osteonecrosis (n=170) (% of patients)	Patients with responses to second questionnaire (n=85) (% of patients)	Significance level (p-value)
Sex			
Male	96 (56%)	44 (52%)	0.55
Female	74 (44%)	41 (48%)	0.55
Ethnicity			
White	141 (83%)	76 (89%)	0.21
Asian	15 (9%)	6 (7%)	0.59
Black	3 (2%)	1 (1%)	0.56
Other	11 (6%)	2 (2%)	0.15
Age (years) at diagnosis of ALL			
<10	22 (13%)	11 (13%)	1
10-15	111 (65%)	57 (67%)	0.75
16+	35 (21%)	17 (20%)	0.85
Treatment protocol			
A	10 (6%)	3 (4%)	0.5
B	108 (64%)	52 (61%)	0.64
C	52 (31%)	30 (35%)	0.52
Number of DI blocks			
1	40 (24%)	19 (22%)	0.72
2	129 (76%)	66 (78%)	0.72
Not specified	1 (1%)	0 (0%)	0.36

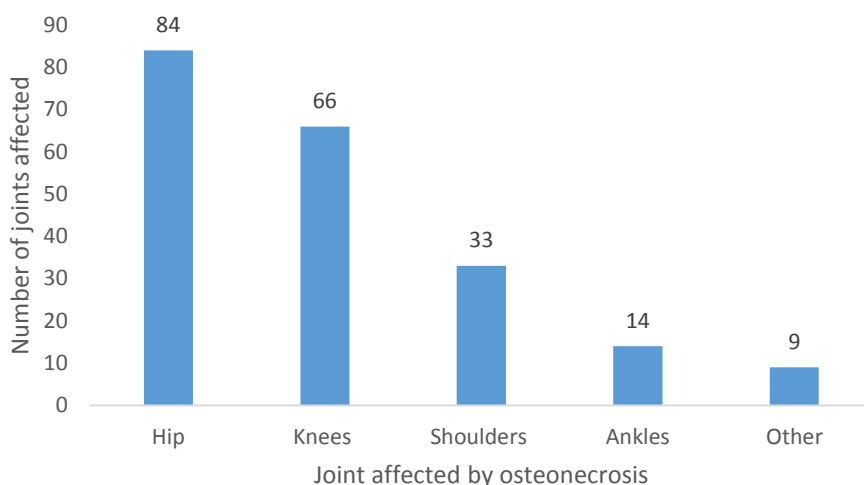
It was reported that 1 patient of the 85 patients with questionnaire responses had bone marrow transplantation- however this information was not specifically requested at time of questionnaire distribution. 3 patients had died at the time of questionnaire data collection (2 died from relapse, one cause of death not specified).

3.4.3.2 Joints affected

In these 85 patients a total of 206 joints were affected. The most commonly affected joints were hips, knees, shoulders and ankles (Figure 21).

Additional areas affected by ON were long bones (n=1), sacrum (n=1), elbows (n=4), wrists (n=2) and metacarpals (n=1).

Figure 21. Joints affected by osteonecrosis in UKALL 2003- surgical sub-study analysis



3.4.3.3 Surgical procedures

Some form of surgical intervention was required in 47% of patients (n=40), with 94 surgical procedures were performed in total. At least one surgical procedure was performed in 33% of all joints affected by ON (n=69), with more than one procedure performed in 8% of joints (n=17). Type of surgery is detailed in Table 27.

Table 27. Surgical procedures performed in joints affected by osteonecrosis

Type of surgery	Number of joints
Hip replacement	36
Shoulder replacement/ resurfacing	4
Knee replacement	4
Core decompression	32
Arthroscopy and debridement	11
Other	8

A total of 43 joints were replaced, with 21% of all joints affected by ON requiring arthroplasty.

Core decompression was undertaken in 32 joints (15%), and was most commonly performed on the femoral head (n=25) (30%). Core decompression was also performed on 4 knees, 2 ankles and 1 shoulder joint. The specific indication for core decompression was not requested from centres, and was not able to be consistently elucidated from clinic letters provided.

There were 30 patients who had arthroplasty as their primary intervention; of these 25 were hip replacements, 3 were shoulder replacements/resurfacing and 3 were knee replacements.

3.4.3.4 Timing of surgical intervention

The median age at first intervention for affected joints was 17.45 years (IQR 15.30-19.60 years).

The median patient age for joint replacement was 18.92 years (IQR 17.33 to 20.17 years), with a median age for hip replacement of 18.38 years (IQR 16.96 to 19.90 years). Joints were replaced at a median of 3.83 years after the diagnosis of ALL (IQR 3.17-4.83 years).

The median patient age at which core decompression was performed was 15.25 years (IQR 13.17 to 17.96 years), which was a median of 2 months after the diagnosis of ON (IQR 1.00 to 7.00 months). The median time for core decompression after the diagnosis of ALL was 20 months (IQR 14.00-30.00 months).

3.4.3.5 Joint specific analysis of interventions for osteonecrosis

3.4.3.5.1 Hips

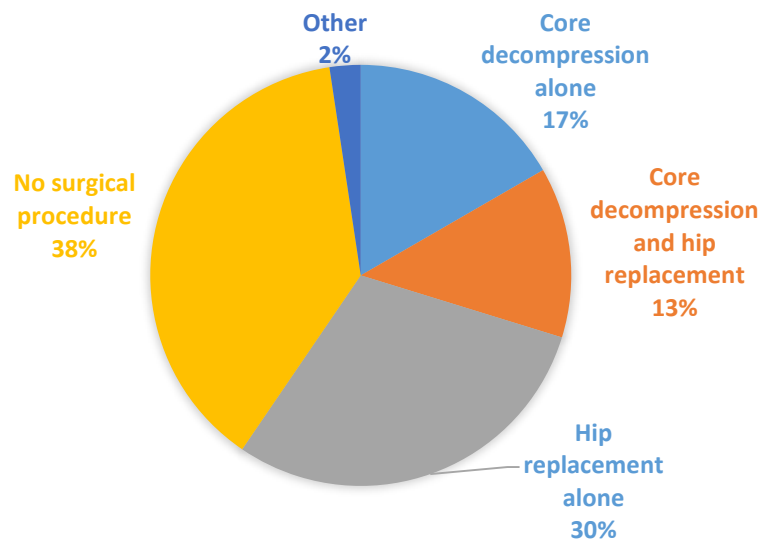
A total of 84 hips were affected by ON, with 25 core decompressions performed (30% of hips affected by ON). One patient with core decompression had shelf osteotomy at the same time as core decompression. The operation notes were available for 16 patients (64%). A pre-operative arthrogram was performed in 1 patient. Four hips had Osteoset® bone graft substitute (25%), and 2 hips had bone marrow aspirate used at the time of the decompression (13%). The most common technique used at the time of decompression was 2 cannulated drill passes. The number of cannulated drill passes ranged from 1 to 5. Of those that had core decompression, 11 hips went on to be replaced (44%).

There was a total of 36 THRs (43% of all hips affected by ON), with 26% of all patients requiring at least one hip replacement (n=22). Of these patients, operation notes were available for 22 patients (61%). Eight different prostheses were used, with one operation note not stating the prosthesis used. The most common prosthesis was an Exeter stem with either a contemporary acetabular component (n= 5, 23%) or a Trident acetabular component (n=5, 23%). The most common fixation technique was a hybrid THR (n=9, 41%), followed by an un-cemented system (n=7, 32%) and then a cemented system (n=6, 27%). The most common bearing surface was ceramic on ceramic (n=14, 64%), followed by ceramic on polyethylene (n=8, 36%).

There was no surgical procedure performed in 33 hips (38%). A small number of affected hips underwent other procedures, some of which went on to THR. These included cheilectomy of femoral head and neck in 1 patient, arthroscopy in 2 patients, excision arthroplasty + pelvic osteotomy in one patient, and femoral lengthening following excision arthroplasty in one patient.

Results are presented in Figure 22.

Figure 22. Surgical management of hips affected by osteonecrosis



3.4.3.5.2 Shoulder

Of the 33 shoulders reported to be affected by ON. Only one had core decompression, and in this case there was no further surgical intervention. Four shoulders were replaced/ resurfaced (12%). No surgical procedure was performed in 28 shoulders. One patient had a hemi-cap fixation and 1 had arthroscopy and debridement, with subsequent replacement.

3.4.3.5.3 Knees

ON affected 66 knees in our cohort of patients, and of these, 4 knees were replaced (6%), and 4 had core decompression as the only surgical intervention (6%). No surgical procedure was performed in 55 knees affected by ON (83%). Other procedures carried out were arthroscopy and removal of loose body (n=3, 5%), curettage and bone graft (n=1, 2%) and reduction and internal fixation of an osteochondral fracture (n=1, 2%).

3.4.3.5.4 Ankles

A total of 14 ankles were affected by ON. Two ankles had core decompression, one of which went on to have arthroscopy and debridement, with no surgical intervention in the remaining 12 ankles (86%).

3.4.3.5.5 Other areas affected

Of the other areas affected, no surgical procedure was performed in an affected metacarpal (n=1), long bone (n=1), sacrum (n=1), or wrist (n=2). Four patients were reported to have ON affecting the elbow. Of these patients, 1 patient had arthroscopy and removal of a loose body and 1 had open reduction and internal fixation of an intra-articular fracture.

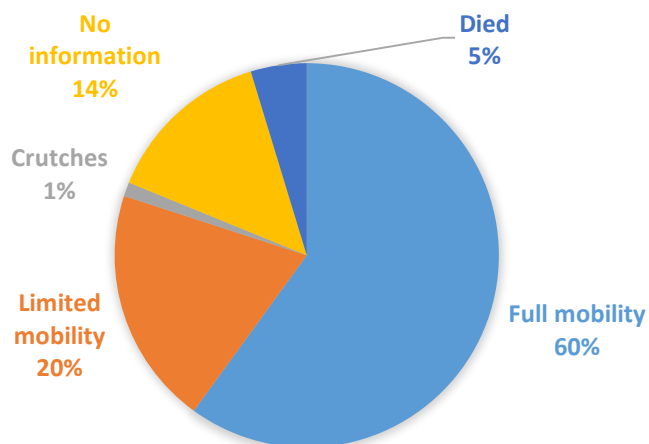
3.4.3.6 Long term outcomes

The outcomes of pain and mobility were assessed by analysis of questionnaire results and data taken from the most recent clinic letters.

3.4.3.6.1 Mobility

Figure 23 illustrates the reported mobility status of patients at a median follow up time of 83 months.

Figure 23. Mobility status of patients with osteonecrosis in UKALL 2003



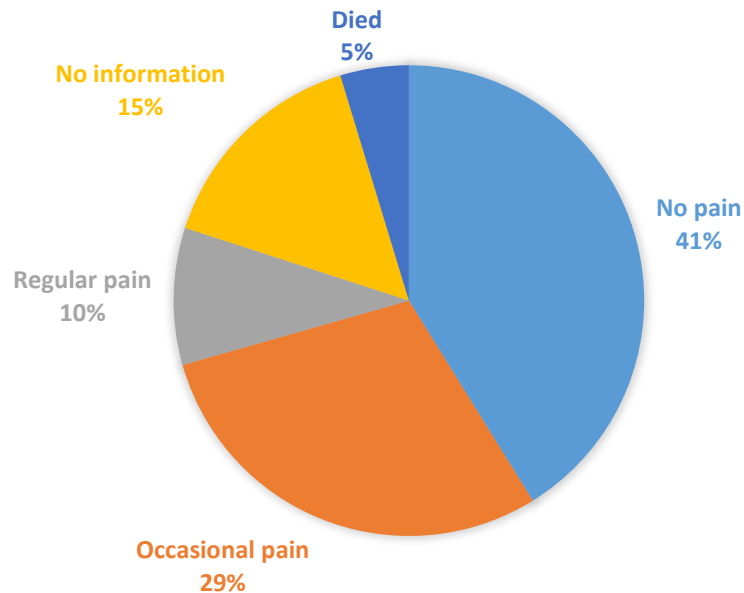
The patient requiring crutches had ON affecting both hips and knees, with no surgical intervention undertaken at the time of data collection.

Of the 17 patients reported to have limited mobility, 8 had had no surgical intervention (47%). Of those with limited mobility, 5 patients were described as having regular pain. Two of these patients had hip replacements, 2 had no intervention and 1 patient had core decompression of the knee.

3.4.3.6.2 Pain

Pain status was also assessed from clinic letters and questionnaire responses. Categorisation of analgesia use was planned, but due to sparsity of data this was unable to be completed. Results are presented in Figure 24.

Figure 24. Pain status of patients in UKALL 2003 with osteonecrosis



It can be seen that 41% of patients reported no pain. Occasional pain was reported in 29% of cases, with only 10% of patients reporting regular pain.

3.4.4 Results: Part B

Efficacy of core decompression of the femoral head

Diagnostic imaging was available for 59 of the 84 hips affected by ON (70%) (35 patients). Of these 59 hips, 20 had core decompression of the femoral head (34%). Median duration of follow up was 6.9 years (IQR: 5.0-8.5 years).

3.4.4.1 Demographics

The median age at diagnosis of ALL in these patients was 14.61 years (IQR: 12.56-16.43 years). 18 patients were female, 17 were male. Ethnicity was defined as White for 30 patients, mixed for one patient and Asian for 4 patients. The median age of diagnosis of ON was 16.17 years (IQR: 14.45-18.09 years), with a median time to diagnosis of ON after diagnosis of ALL of 1.17 years (IQR: 0.96 to 1.68 years). In those patients who had core decompression, the intervention was performed at a median of 3 months after the diagnosis of ON (IQR: 1-7 months).

Results of grading of hips along with outcomes are presented in Table 28.

Table 28. Grading of hip osteonecrosis at diagnosis

Osteonecrosis grade at first imaging	Number of hips at given grade of osteonecrosis (% of all hips)	Number of hips with core decompression (% of hips at given grade)	Number of hips to reach grade 5 ON/ THR (% of hips at given grade)	Number of hips to reach grade 5 ON/ THR after core decompression (% of hips with core decompression at given grade)
2	2 (3%)	2 (100%)	2 (100%)	*
3	7 (12%)	1 (14%)	2 (29%)	0
4	37 (63%)	13 (35%)	27 (73%)	8 (62%)
5	13 (22%)	4 (31%)	Not applicable	Not applicable
Total	59	20	44	10

*although 2 patients had grade 2 ON at initial MRI, the core decompression only occurred after the patients had progressed to grade 5 ON.

The majority of hips were grade 4 or 5 at diagnosis of ON (86%).

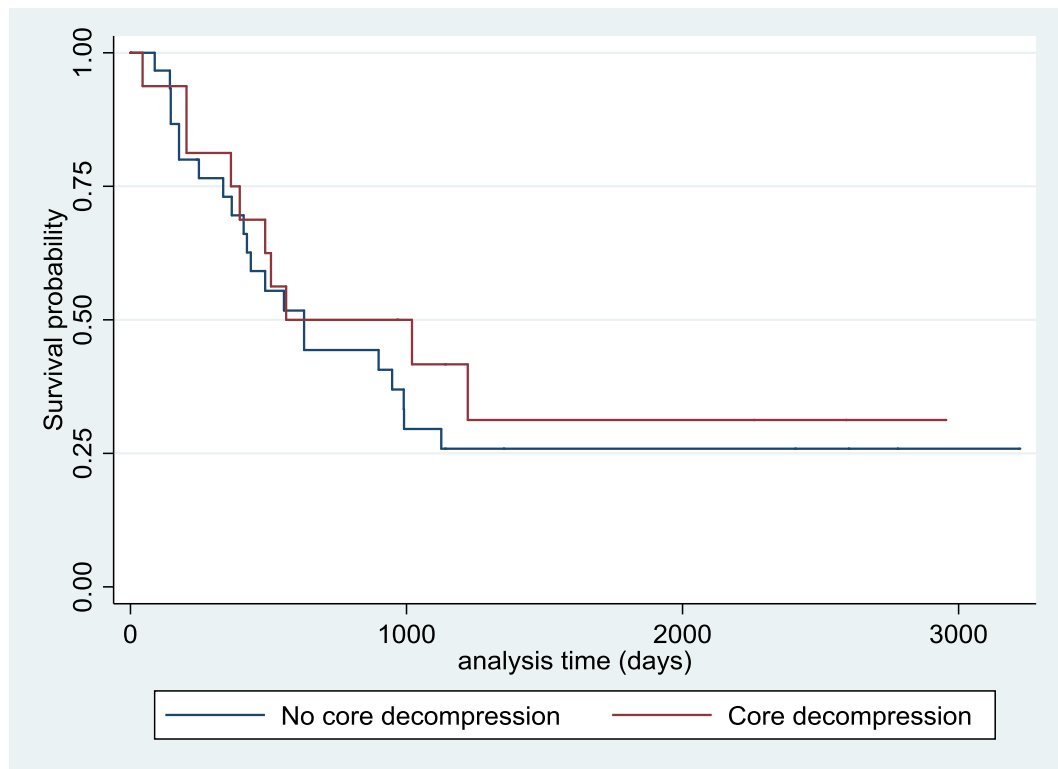
The total failure rate in terms of joint collapse for the study group was 75%.

At time of decompression there was a median of 2 cannulated drill passes, with a range of 1 to 5 drill passes (IQR 1.8-3). 2 hips had Osteoset® bone graft substitute used at time of core decompression, and both of these hips also had bone marrow aspiration. The two hips where Osteoset® bone graft substitute was used were grade 4 at diagnosis of ON, and had a survival time of 203 days.

3.4.4.2 Survival analysis

A Kaplan-Meier failure time estimates comparing core decompression with no early surgical intervention in patients with ON of the hip is shown in Figure 25.

Figure 25. Kaplan-Meier failure time estimates for hips comparing core decompression with conservative treatment.



Survival time: time since diagnosis of osteonecrosis to reach grade 5/ total hip replacement (whichever sooner)

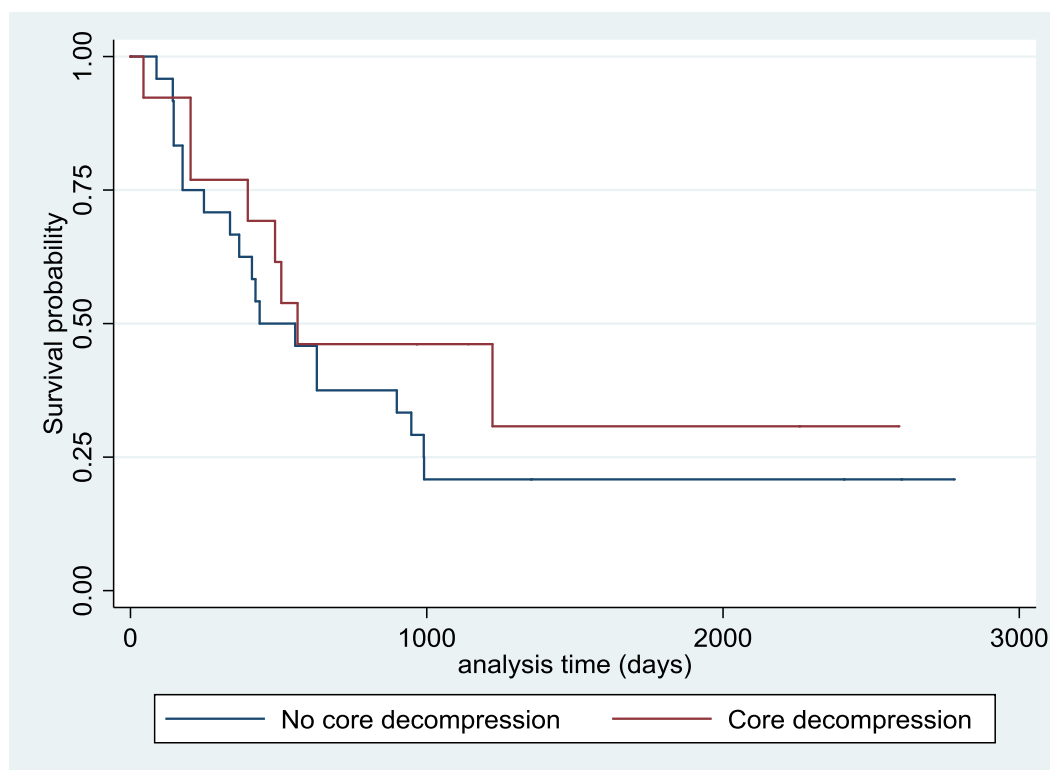
Event status: event=THR/collapse, censored=no collapse

The median time to develop joint collapse/ THR for the patients who had core decompression (excluding those with grade 5 ON at diagnosis) (n=16) was 765 days (IQR 388-1161 days), compared with 522 days for those who had no joint preserving surgical intervention (IQR 270-1092, n=30). Cox regression showed no significant difference between the two groups (hazard

ratio=0.79, $p=0.57$, 95% CI= 0.34 to 1.82), although core decompression was associated with a 20% lower risk of joint failure compared with conservative treatment.

The majority of patients in this analysis had late stage ON (grade 4 or 5) at diagnosis. The patients with grade 4 ON were analysed as a subset in an ad-hoc subgroup analysis. The Kaplan-Meier survival curve is presented in Figure 26.

Figure 26. Kaplan-Meier failure time estimates for hips with grade 4 osteonecrosis comparing core decompression with conservative treatment.



Survival time: time since diagnosis of osteonecrosis to reach grade 5/ total hip replacement (whichever sooner)

Event status: event=THR/collapse, censored=no collapse

In this subset of patients, the median time to event (THR/grade 5 ON) for the patients in whom core decompression was performed was 442 days (IQR 203-523 days), compared with 410 days for the conservatively managed group (IQR 176-629 days). Cox regression indicated no significant difference between the groups (hazard ratio= 0.69, $p=0.42$, CI= 0.28 to 1.69) although the hazard ratio suggests core decompression in this patient group was associated with a 30% lower risk of joint failure compared with conservative treatment.

Chapter 4 The British OsteoNEcrosis Study

4.1 Study development

4.1.1 Introduction

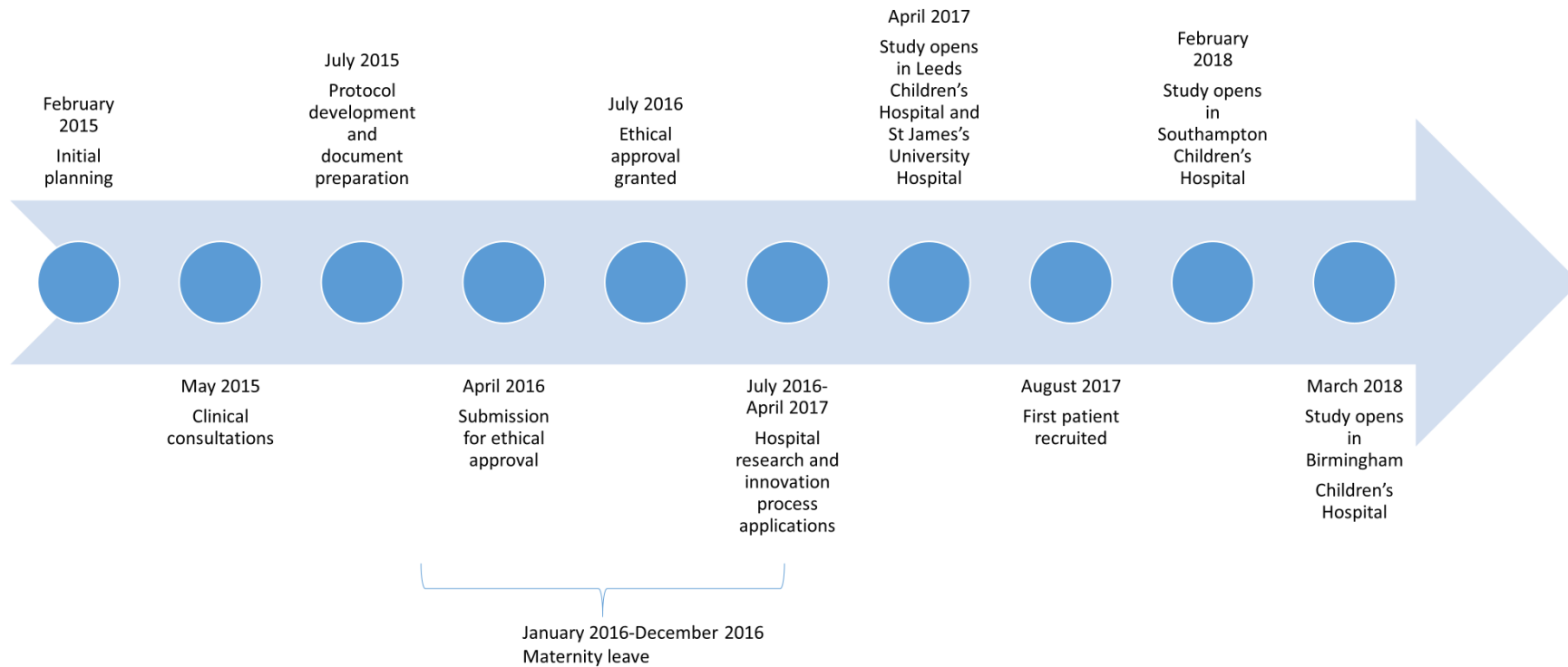
It is clear from Chapters 2 and 3 that there is a need for a greater understanding of the factors affecting the development of ON in children and young people treated for ALL and LBL.

This chapter describes the protocol development for the British OsteoNEcrosis Study (BONES), a prospective cohort study developed to examine the natural history of ON in older children, teenagers and young adults with ALL and LBL. The first part of this chapter describes the rationale, feasibility assessment and multidisciplinary involvement in developing the study methodology. The second part of this chapter describes the final study protocol. The last section reports our preliminary results. A discussion of the results will be presented in Chapter 5. At time of thesis submission the study is ongoing.

The complete study protocol is provided in Appendix 10.

A timeline of the work for the study is shown below in Figure 27.

Figure 27. Timeline of events for development of British OsteoNEcrosis study



4.1.2 Consultation phase

This study arose due to an increasing awareness of the significant morbidity associated with ON in young people with ALL, both within paediatric haematology, and in patient and carer groups [1]. Initial discussions about the proposal for a prospective longitudinal study took place within a core group comprising of clinicians from tertiary paediatric haematology departments, and was further developed by a national toxicity working group focussed on ON. There were concerns that there was incomplete understanding of the pathophysiology and natural history of osteonecrotic lesions, and a consensus view that a UK study specifically targeting young people with ALL would be of value.

Once a decision had been made to develop the study, I was involved in a series of meetings with professionals and families, which allowed the formal development of the study protocol.

4.1.2.1 Initial consultations with professionals

For this part of the consultation phase I discussed different elements of the study protocol with a range of health professionals. This included representatives from paediatric haematology, endocrinology, orthopaedic surgery, radiology, and physiotherapy. In the development stages the study concept was also presented at national ALL toxicity working group meetings and physiotherapy meetings to allow for discussion of the most appropriate methodology. These presentations provided an opportunity to discuss some of the issues facing clinical and research departments across the country. My initial consultations enabled me to understand feasibility, scientific validity and clinical concerns of the different professionals involved in the care of patients with ALL. Some important areas for consideration were resource capacity, methods of minimising study burden to patients, and optimal timing of discussions about study participation with patients and families. The outcomes of these meetings facilitated the development of the study aims and objectives, as well as the initial protocol and assessment processes.

4.1.2.1.1 Radiology

The involvement of a paediatric radiologist specialising in musculoskeletal radiology was essential. During the consultation phase the main issues discussed with radiology, together with paediatric orthopaedic and endocrine input were:

- Feasibility of MRI in paediatric patients, including length of time of scan and need for sedation/ anaesthesia
- The most relevant body areas to image
- Frequency and timing of imaging
- Management of MRI results
- Incorporation and use of DXA and VF assessment

4.1.2.1.2 Physiotherapy

The physiotherapy assessment was developed in collaboration with the paediatric physiotherapy team at Leeds Children's Hospital, who had an existing interest in the management of ON in patients being treated within the paediatric haematology and oncology service. The plans were also discussed at a national physiotherapy meeting. Whilst developing the protocol, it became clear that this study provided an opportunity to incorporate a physical assessment of the patient which could be correlated with radiological and biochemical data. It was important to determine current practice both locally and nationally, and to identify methods of patient evaluation. The main issue was the lack of a validated paediatric ON assessment tool. A range of subjective and objective assessment methods were assessed, to identify patient evaluation tools which would be acceptable and suitable for our patient population and which would aim to identify early signs or symptoms of ON.

4.1.2.2 Consultation with families

Once there was broad agreement over the study design and development of study literature, I conducted a series of structured discussions about the study with patients and families diagnosed with ALL. The consultations comprised of informal meetings with individual patients and families to review and discuss the proposed study design and study literature.

Patients and families with ALL were approached in the paediatric haematology day unit, and discussions were held in side rooms prior to or after their clinical appointment. Six families were contacted, with an equal mix of male and female patients, and a range of patient ages. Discussions lasted between 30 to 45 minutes, and included the following points:

- An explanation of the study aims
- An explanation of study design and patient involvement
- Review of study paperwork, including patient and parent information leaflets
- Whether the young person and parents would have felt happy to participate in the study

There was also the opportunity to discuss additional issues or concerns the family had regarding the study.

4.1.3 Key points emerging from consultations with professionals

4.1.3.1 Patient population

Previous research has repeatedly found that patients with the highest risk of development of ON were those over 10 years of age at diagnosis of ALL. It was felt to be appropriate to target these patients as the group most likely to develop significant morbidity from ON, as well as a group of patients likely to be able to tolerate imaging without additional sedation or anaesthesia.

Patients with both ALL and LBL were included within the study as both groups currently receive the same chemotherapeutic treatment, and hence have the same risks of treatment toxicity. The upper age limit of 24 years was chosen as it correlates with the upper age limit for inclusion within the current national study for children, teenagers and young people with ALL or LBL (UKALL 2011). Although it has been shown that the incidence of ON reduces in patients over 20 years of age, it is still significantly higher than in patients under 10 years of age at ALL diagnosis [286], and this is a group of patients often overlooked in research studies.

It was decided that the only essential exclusion criteria was an inability to tolerate the study investigations.

The recruitment target was developed using the retrospective data from UK centres, described in Chapter 3, which enabled us to predict incidence of patients diagnosed with ALL/LBL aged 10-24 years.

4.1.3.2 Imaging

MR imaging was chosen as it provides a non-invasive diagnostic evaluation of a region of interest, and is more sensitive in the detection of early stage focal ON than CT or plain radiographs, even with limited MR imaging protocols [288-291]. MRI images clearly depict size of lesions and allows sequential evaluation of asymptomatic lesions that are undetectable on plain radiographs [164]. Contrary to most other imaging modalities which might detect ON, MRI does not use ionising radiation, which is of particular significance in the vulnerable growing skeleton. MRI is also capable of imaging in multiple planes, and has high spatial and contrast resolution, allowing evaluation of morphological features [292].

Although there was an initial desire to image both upper and lower limbs, a more pragmatic approach prevailed. This took into consideration access to MRI, cost of imaging and time to scan. It was decided that MR imaging of

lower limbs was of greatest value, given the preliminary results presented in Chapter 3 highlighting the high prevalence and morbidity associated with ON affecting hips and knees. It is possible to scan lower limbs in a single 30 minutes assessment, limiting both cost and time in scanner for patients. Non-contrast coronal T1-weighted spin-echo and short tau inversion recovery (STIR) sequences (which nulls the signals from fat) were determined to be sufficiently detailed to determine presence or absence of osteonecrotic changes in hips, knees or ankles [292]. Although the use of intravenous contrast highlights areas of decreased enhancement in the necrotic bone and increased enhancement at the reparative surface, this can be differentiated without use of contrast as viable tissue exhibits low signal intensity on T1-weighted and intermediate or high on STIR MR images, whereas necrotic areas are hypo-intense on all sequences [293]. This avoids the need for intravenous access and administration of potentially allergenic contrast.

When assessed by MRI, ON is visualised as an area of yellow marrow surrounded by a low signal intensity rim on all pulse sequences or a double line rim comprising of a low signal line and an adjacent high signal line on fluid sensitive sequences. The area of ON may be complex in shape with serpentine, crescentic, band-like or undulating outline or represented as multiple small lesions [294-296]. It was also decided that non-classical abnormalities would also be recorded if encountered, including haemorrhagic or cystic change as well as non-specific marrow changes and marrow oedema, as these have been previously described and may represent a significant prognostic factors for development of ON [294-296].

In Chapter 2.1.4 the advantages and disadvantages of various ON scoring systems were highlighted. After discussion, it was concluded that the classification system published by Niinimäki et al to assess ON was the most suitable system to use in this study [167]. As this system is not joint specific it can be used to assess hips, knees and ankles. A radiology proforma was developed by the consultant radiologists involved which enabled them to separately record ON seen within the metaphysis and diaphysis of long bones. If different scores were seen for two bones comprising a joint (e.g. tibial and femoral epiphysis as part of the knee) both scores were captured before giving the overall score for the knee, with the aim of assessing the overall burden of ON in the lower legs.

Within the haematology community there were significant concerns regarding the management of information obtained from the MRI scans. The

greatest issue was the need to ensure that there was no change in the management of patients recruited to the study, and that clinicians did not find themselves in a position where they had information about osteonecrotic lesions which they had not solicited.

Consequently, the protocol was clarified to make it clear that the images were not routine MRI scans, and were not for local interpretation. It was decided that local reports would simply say “images are for trial purposes only”. In line with common study practice, if a significant abnormality other than ON, such as a fracture, was found when images were centrally reviewed, information was to be fed back to the local centre. In the event of the development of locally diagnosed symptomatic ON, the patient was to be managed according to local protocols and at the discretion of their own consultant. At present, the optimal management of asymptomatic ON is not known, and so there would be no benefit for an asymptomatic patient to be made aware of osteonecrotic changes.

Determining the optimal timing of imaging was of considerable importance. The first time-point was chosen to be as early as practically possible, to assess if there were any early changes that may indicate the likelihood of a patient developing extensive ON. It was initially planned that patients would be assessed within 2 weeks of the diagnosis of ALL. However, it became apparent that there were practical difficulties in consenting patients and then organising imaging within this timeframe, resulting in poor recruitment of patients. A major amendment was submitted and approved, increasing the initial window for consent, imaging and assessment from 2 to 4 weeks of diagnosis. This significantly improved study recruitment.

The end of DI was chosen as the second time point for assessment, as by this point patients have had the majority of their high dose steroids, widely thought to be a causative agent. Previous studies have shown the majority of lesions develop early in treatment [132], and it was hoped that an assessment at this point would ascertain the presence of most emergent lesions.

In order to determine the natural history of osteonecrotic lesions there are 3 further annual assessment points. These provide the opportunity to assess progression or regression of lesions, as well as the onset of signs and symptoms in patients.

During the course of the study development there was an increasing awareness of the high frequency of VFs in patients with ALL [196]. The

decision was made to include analysis of annual DXA scans and VF assessment as a bone health adjunct. In some centres this has become standard of care for patients, but there is significant variability across the country. Lateral vertebral assessment of patients using DXA scans was felt to be the most suitable method of assessment of vertebral fractures due to the low radiation exposure to patients and high level of accuracy in the assessment of VFs [44, 45], although lateral spine radiographs are an acceptable alternative in centres without DXA imaging facilities.

Consideration was given to the need to standardise DXA results, and following consultation with paediatric endocrinology and radiology it was determined that the most valid method for this was using the amalgamated reference data for size-adjusted bone densitometry measurements reported for UK children and young people in the ALPHABET study [38]. This provides a method for calculating size adjusted results, with lumbar spine BMAD (g/cm^3) and total body less head (TBLH) the most valuable measurements for analysis [32].

In order to reduce observer error a central review panel of paediatric radiologists with an interest in paediatric haematology was established. Each MRI was to be assessed by the panel in order to agree the grade of ON according to the radiology proforma. In addition, DXA and lateral vertebral assessments would be assessed centrally using the Genant semi-quantitative method. This was chosen as at the time of study development it was the most widely used method for diagnosis and grading of vertebral fractures [47].

4.1.3.3 Clinical and demographic information

It was clear from the existing literature that age and sex of patient were important demographic details to capture. Height and weight were included within demographic data collection to enable calculation of BMI SDS and height velocity of patients during treatment. BMI SDS, rather than BMI, was chosen for use as BMI was initially developed and applied to adults as a correlate of adiposity. For children, BMI varies with age, not only with weight. Because of this, BMI values will be compared with reference values that are age and sex specific, and transformed into a standard deviation score (Z-score) [297].

Following our collection of retrospective data from UKALL2003, and given the paucity of high quality literature in this area, the value of collecting ethnicity data was recognised. Following discussions around other possible

causative factors for development of vertebral fractures and ON, postcode collection was also incorporated into the data collection form to allow analysis of deprivation score of participants. The index of multiple deprivation (IMD) is calculated using the index of multiple deprivation 2015. This ranks every small area in England from 1 (most deprived) to 32,844 (least deprived) [298].

4.1.3.4 Pubertal assessment

Given the increased risk of ON in patients of pubertal age, it was important to incorporate an assessment of puberty of patients participating in the study. Tanner staging is the most widely used detailed method of pubertal assessment, and is an objective classification system that allows tracking of the development and sequence of secondary sex characteristics of children during puberty [299, 300]. However, it requires considerable user experience and is an examination which should only be performed with a chaperone present. It was clear from discussions that it is rarely carried out by paediatric haematologists, and consultations with clinical staff highlighted concerns about this method of assessment. A collective decision was made to use the simplified form of pubertal staging which is used by the Royal College of Paediatrics and Child Health in the childhood and puberty close monitoring growth chart. In this, phase of puberty is assessed by questions rather than an intimate examination, and patients are categorised into 3 phases: pre-puberty (Tanner stage 1), in puberty (Tanner stage 2 and 3) and completing puberty (Tanner stage 4 and 5) [301]. Although this provides less detailed information than the Tanner stage, it can be ascertained through simple questions about the presence of secondary sexual characteristics and pubertal milestones.

4.1.3.5 Biochemical data

As one of the aims of this work was to identify risk factors for development of ON in ALL/ LBL, data collection needed to include diagnostic and prognostic indicators for the condition being treated. Therefore data about the individual's immune-phenotype, cytogenetics, molecular results, flow cytometry and MRD status were to be collected.

Previous literature has suggested that changes in the lipid profile and albumin levels affect the risk of ON development [132], and these were incorporated into our data collection. Initially, we planned to collect information on specific markers of bone turnover. These are not routinely used in clinical practice and markers considered included carboxy-terminal

collagen crosslinks (CTX), bone specific alkaline phosphatase and N-terminal pro-peptide of type 1 procollagen (P1NP). During ongoing discussions with specialists in metabolic bone medicine, it was felt that the study would be insufficiently powered to draw meaningful conclusions from these results, and as these tests were not routinely used in clinical practice there would be little benefit in collection of these data. The tests also placed an additional burden on patients, and would potentially prevent patients from participating in the study. After consideration, these were removed from the study as part of a major amendment, and replaced by collection of PTH, vitamin D, calcium and phosphate results. These are tests that are routinely performed and used in clinical practice, and hence were felt to be likely to provide more clinically valuable data, without placing additional demands on the patient.

4.1.3.6 Physiotherapy assessment

The physiotherapy assessment facilitates integration of biochemical, radiological and clinical information. It was established that in Leeds all patients with a new diagnosis of ALL had a baseline subjective assessment of pain and current levels of activity, along with gait assessment and discussions about maintaining mobility during treatment. When this was discussed in national physiotherapy meetings it became clear that such an assessment was not universal. In the majority of other centres physiotherapy assessments occurred only if a specific referral was made due to physician concerns. Once this was recognised, it was clear that a standardised approach to patient assessment would be required for this study.

There was value in collection of both subjective and objective data during this assessment. Subjective data allowed an understanding of the patient experience, whilst objective data collection aimed to help to determine physical signs that may relate to ON development. It was decided that a questionnaire would be the best method of subjective assessment. Patient questionnaires are a form of outcome measure that are universally accepted, and a way of collecting data quickly and efficiently. If correctly phrased and formatted they are able to provide a subjective, patient-centred approach to outcome evaluation. Using subjective and objective tests in combination helps to remove bias that comes from only having a single perspective [302]. The areas we wished to assess were activity levels, mobility, and pain.

The ideal questionnaire would be one which:

- Is validated in our population,
- Is age appropriate
- Assessed the relevant domains
- Has internal consistency
- Is easy to complete and acceptable to patients.

A number of different questionnaires were evaluated for suitability. These included the:

- Quality of life Evaluation in patients receiving Steroids (QuEST) [303]
- Lower Extremity Functional Scale (LEFS) [304]
- Juvenile Arthritis Functional Assessment Scale (JAFAS) [305]
- Activities Scale for Kids (ASK) [306]
- Paediatric Outcomes Data Collection Instrument (PODCI) [307]
- Pediatric Quality of Life Inventory (PedsQL) [308]
- Childhood Health Assessment Questionnaire (c-HAQ) [309]

The only questionnaire validated for use our population was QuEST, an assessment tool developed to assess the quality of life in patients aged 8-24 years receiving maintenance therapy for ALL. However, many of the questions were not relevant in our setting, with only one of the four domains assessing physical health (other domains were appetite and body image, emotion and cognitive effects) [303].

The LEFS was not used in BONES due to the lack of validation in the paediatric population, with some questions that were unsuitable for use in this setting. The JAFAS is used for patients aged 7-16 years, was specifically developed for children with rheumatoid arthritis, and requires observation of the child's performance of activities under standardised conditions. This was not a suitable assessment tool for our study due to the narrow age range and the training required to administer the test. In addition, there is no published evidence that the JAFAS is able to detect change in a child's physical function over time. The ASK and PODCI assess physical function in children with chronic health disorders. The ASK is designed only for assessment of children aged 5-15 years, and requires a licence for use. These factors made the use of the ASK unfavourable for use in our study. PODCI is an assessment tool for patients aged 2-18 years, and was developed to evaluate problems related to bone and muscle conditions. There is no UK version of the questionnaire, and as scoring is calculated using knowledge of the general population mean (standardised) score and corresponding standard deviations, it was felt not to be applicable for our patient population.

The PedsQL can be completed by young people aged 5-18 years, and assesses physical, emotional, social and school functioning. There were a number of questions that were too non-specific, particularly those around feelings, school and social functioning, and therefore the PedsQL was discounted for use in BONES.

The c-HAQ was developed for children with rheumatoid arthritis [310], but has since been validated in a number of different patient populations. One comparative study of different measures of paediatric function compared 5 different measures of paediatric function, including the c-HAQ, JAFAS, PODCI and ASK [311]. When these were all compared only the c-HAQ was found to have excellent validity and reliability and good responsiveness. Although this was in patients with juvenile arthritis and juvenile idiopathic inflammatory myopathies, the c-HAQ evaluates health status and physical function, and assesses a child's capability to perform activities in their daily environment. It is validated for use in young people with juvenile arthritis [310], chronic musculoskeletal pain [312], dermatomyositis [313] and systemic lupus erythematosus [314]. It includes the international classification of functioning, disability and health components of body function and activities and participation, as well as a measure of overall health status. It was important to consider the respondent burden and usability. The cultural English language version of c-HAQ takes 10-15 minutes to complete, with language that is simple and easy to read. A disadvantage of use of the c-HAQ is its ceiling effect in children with mild disease. This means that it is challenging to measure improvements at the better end of the functional spectrum (i.e. clinical validity is reduced), but given the reliability (internal consistency (Cronbach's coefficient α) of 0.94) and ease of use it was felt to be the most suitable questionnaire for use in our study [315]. Further validation of this questionnaire in our specific patient population would be of value, but is out of the remit of this study given the anticipated sample size.

Together with the c-HAQ it was felt that a subjective assessment of pain in specific areas was required, and the Wong-Baker Faces pain scale was used as a self-report measure of pain intensity developed for children [316]. This allows the scoring of pain on a widely accepted 0-10 metric. Permission was applied to use the scale within our study and was granted by the Wong-Baker FACES Foundation.

The objective assessment was developed by paediatric physiotherapists. The aim was to develop a physical assessment evaluating gait, range of

movement and muscle power which could easily be replicated across centres.

4.1.4 Development of study literature

An essential element of the development of the study protocol and submission for ethical review was the design of study literature. This included development of patient information leaflets, parent information leaflets, consent and assent forms, and data collection forms. Consent and participant information sheets were prepared in line with the Health Research Authority and Medical Research Council guidance [317]. It was felt that the age range of 10-24 years was too broad for a single patient information leaflet, and hence different leaflets were developed for patients aged between 10-12, 13-15 and over 16 years. These varied in level of detail to ensure they were suitable for patients at different developmental stages.

As well as explaining why, how and where the study was being conducted, the information sheets included information about the study website and details of a local contact for further information. A letter was also developed to send to families after their discharge from hospital to thank them for their involvement and to remind them of the next stage of the study. A sample of study literature is available in Appendix 11.

Data collection forms were developed in collaboration with the healthcare professionals involved in research data collection.

4.1.5 Study development following consultations with families

The consultations with families identified overwhelming support for a study looking at ON. Patients and families, particularly in the later stages of treatment, recognised it as an important complication requiring further research.

Of the 6 families approached, 5 felt they would definitely have participated in the study. The sixth family had concerns that their child would not be able to tolerate the MRI scans, and hence would not be able to participate.

Patient and families were generally happy with the study literature, which was felt to be age appropriate for the different patient groups. Minor amendments were made to wording.

4.1.6 Study promotion

4.1.6.1 Communicating with healthcare professionals

A website was developed with the assistance of IT support from the University of Leeds for use by healthcare professionals and patients. This was to act as a gateway to information for healthcare professionals, including access to all documents required for the study, as well as a source of additional information for patients and families. As the website was built on a University of Leeds platform, there were limitations to site design but this format had the advantage of on-going IT support. The study was discussed at regional, national and international meetings for paediatric physiotherapy, haematology and endocrinology. This stimulated significant interest in participation and study development. To maintain interest in the study I sent regular newsletters to all participating and interested centres with updates on study progress and explanations of any developments.

4.1.6.2 Communicating with families

The website described above provided patients and families with current study information. Families were also sent thank you letters after they returned home, to express our gratitude for their participation.

4.1.7 Data management strategy

Each patient is allocated a unique identifier at enrolment to the study. A Microsoft Access database was developed to use this identifier as the primary key, allowing recording and linking of all the socio-demographic and clinical data for a study participant with information from their radiology assessments. An Access database was chosen as it is a relational database management system enabling easy storage of information for reporting and analysis.

In order to comply with data protection regulations it was determined that data would be submitted centrally via a secure NHS email address with all patient identifiers removed. At each hospital site local clinicians and physiotherapists completed the relevant forms at each time-point, with forms anonymized locally. Images of MRI scans and DXA images were anonymized locally and placed onto CDs which were sent to the central trial unit.

4.1.8 Statistical analysis

Data was planned to be collected and analysed in clinically relevant categories, and analysed as continuous variables when possible.

The full dataset was to be analysed using Chi-squared tests and multivariable logistic regression models, to determine differences between groups adjusting for a relevant set of confounders identified using causal inference methods [283]. Potential confounders to be assessed include age, sex, ethnic group, IMD, treatment arm, highest white cell count, immune-phenotype, cytogenetics, phase of puberty, body mass index z-score, lipids, albumin, VFs, BMD, ALP, PTH and vitamin D status. If numbers are sufficiently robust a more sophisticated ordered logistic regression analysis was planned to be carried out using an ordered categorical outcome variable for severity of ON.

Discussion with epidemiologists established that the optimal use of data was to use an intention-to-treat principle in the analysis. It was decided that if data on some subjects were missing at some time points the entire subject history will not be excluded from analysis. If the data were missing at rates higher than the expected attrition rate the following steps were to be taken:

- If data regarding independent variables were missing but data for the corresponding dependent variables are present, we would do multiple imputations for the missing values
- If some data associated with a dependent variable were missing, such as some follow-up data, and the underlying mechanism is random, only the missing observations were excluded
- If some dependent variable data were missing and the underlying mechanism was not random, we will estimate group effects according to methods proposed by Wu and Bailey [318] and Milliken and Johnson [319]

4.1.9 Summary of amendments

After completion of the initial consultation and development phase of the study, there have been further discussions with different healthcare professionals and families to allow evolution of the study as new issues came to light. The main modifications that were incorporated into major amendments are as follows:

- The development and promotion of the British OsteoNEcrosis study website
- Change of physiotherapy questionnaire to c-HAQs, as discussed above
- Removal of measurement of P1NP, CTX and bone specific alkaline phosphatase
- Extension of the time to first assessment to 4 weeks, to allow patients longer to assimilate and understand the study prior to consent
- Incorporation of collection of ethnicity and postcode data

The next major amendment, which will be submitted shortly will include the following exclusion criteria:

- Patients diagnosed with mature B-ALL (Burkitt-like, t(8;14), or C-MYC rearranged regardless of morphology or phenotype)
- Patients diagnosed with Philadelphia-positive ALL (t(9;22) or BCR/ABL positive)
- Patients who fail induction treatment. If patients are recruited but subsequently fail induction they should be withdrawn, with no further assessments

This is because all of these patient groups will receive significantly different treatment regimes, limiting the value of comparison between patients.

4.2 The British OsteoNEcrosis Study Protocol

4.2.1 Objectives

The objective of this study was to establish a prospective, multi-centre study for older children, teenagers and young adults to address the following questions:

- What is the incidence of symptomatic and asymptomatic ON in older children, teenagers and young adults being treated for ALL or LBL in the UK at different time points in their treatment?
- What are the risk factors for progression and the development of symptomatic ON in this population?
- Are there specific radiological features that predict for either progression or regression in those with asymptomatic ON?

The study also aims to:

- Evaluate functional ability and explore the correlation of this with MRI findings
- Evaluate changes in BMD and VF incidence during treatment for ALL or LBL

4.2.2 Study design

BONES is a prospective longitudinal cohort study.

4.2.3 Study setting

The study is being conducted in PTCs and teenage and young adult centres for patients with cancer within the UK. It is currently open in Leeds Children's Hospital; St James's University Hospital, Leeds; Birmingham Children's Hospital; and Southampton Children's Hospital.

4.2.4 Study population

Inclusion criteria: children, teenagers or young adults between the age of 10 and 24 years 364 days (at the time of diagnosis) with a first diagnosis of ALL or LBL (TNHL or Smlg negative precursor B-NHL) diagnosed under standard criteria.

Exclusion criteria: inability to have MRI scans of lower limbs

4.2.5 Recruitment target

The recruitment target is 50 patients over a 3 year period, which was based on an anticipated participation of 75% of eligible cases. Given the observational nature of the study, the emphasis on hypothesis generating, and the wide number of potential predictors of interest, a power calculation was of limited relevance.

4.2.6 Study outcomes

Primary outcome:

- Cumulative incidence of symptomatic and asymptomatic ON in patients aged between 10 and < 25 years being treated for ALL or LBL in the UK at multiple time points in their treatment

Key secondary outcomes:

- Risk factors for progression and development of symptomatic ON
- Specific radiological features that predict for either progression or regression in those with ON
- Evaluation of functional ability as measured by c-HAQ and physiotherapy assessment and exploration of correlation of with radiological findings.
- BMD changes as measured by DXA during treatment for ALL or LBL
- Prevalence and risk factors for development of VFs during treatment for ALL or LBL

4.2.7 Patient assessment

4.2.7.1 Radiology assessment

Irrespective of symptoms patients were screened for ON via prospective MRI of the hips, knees and ankles at the following time-points:

- Within 4 weeks of diagnosis
- At the end of DI (typically 6 to 8 months after start of ALL treatment)
- One year after the start of maintenance treatment

- Two years after the start of maintenance treatment
- Three years after the start of maintenance treatment

MRI of the lower limbs comprised of unenhanced coronal T1 weighted and STIR (short tau inversion recovery) images of 5mm (or less) slice thickness as a minimum protocol. Scanning parameters varied slightly depending on available MR scanners in each participating centre.

DXA scans and vertebral fracture assessments were performed at diagnosis of ALL, and annually for 3 years after diagnosis, and assessed posterior-anterior lumbar spine (L1-4) and TBLH areal bone mineral density, and thoracic and lumbar vertebral fracture incidence.

4.2.7.2 Clinical and demographic data collection

Baseline demographic data collection included the child's age, sex, ethnic background (White British; Asian; Black; Mixed; Other), postcode, height and weight at diagnosis. Clinical data were provided by the treating clinicians via a dedicated clinical report form, which included information on pubertal status, highest WCC prior to treatment, immunophenotype, cytogenetics and molecular results, along with presence or absence of hepatomegaly, splenomegaly, lymphadenopathy and bone pain at diagnosis.

At each of the time-points outlined above details regarding treatment regime, height, weight, phase of puberty, and diagnosis and management of symptomatic ON were collected. Data on results of routine blood tests, including lipid profile, albumin, bone profile, PTH and vitamin D levels were collected. Clinicians collecting these details were blinded to the study MRI reports.

4.2.7.3 Physiotherapy evaluation

Patients had a subjective and objective physiotherapy assessment at each of the same time points as MR imaging (within 4 weeks of diagnosis of ALL, at the end of DI and annually thereafter, to a total of 5 assessments). The physiotherapy assessment consisted of a paper questionnaire for completion by the participant, which included information about activity levels, mobility, pain and the c-HAQ, [310] alongside a physical assessment evaluating gait, range of movement and muscle power.

A schema with BONES study procedures is presented Figure 28.

Figure 28. Schema of British OsteoNEcrosis Study procedures

	Within 4 weeks of diagnosis	Week 1-4	End of induction	End of delayed intensification	One year after diagnosis	One year after start of maintenance	Two years after diagnosis	Two years after start of maintenance	Three years after diagnosis	Three years after start of maintenance
Consent	◇									
MRI scan lower limbs		◇		◇		◇		◇		◇
Routine blood tests (LFTs, calcium, phosphate, cholesterol, triglycerides, HDL, LDL Vitamin D, PTH)		◇	◇	◇		◇		◇		◇
Physiotherapy assessment including c-HAQ		◇		◇		◇		◇		◇
DXA scan with vertebral fracture assessment		◇			◇		◇		◇	
Clinician assessment		◇		◇		◇		◇		◇
End of induction form			◇							

4.2.8 Data analysis

Data was collected and analysed in clinically relevant categories.

4.2.8.1 Demographic and clinical data

Where possible, both demographic and clinical data were analysed as continuous variables.

Cytogenetics and molecular results were coded into 3 groups: high risk; intermediate risk; and good risk. These are as follows:

- High risk - these are cytogenetic and chromosomal abnormalities that have been associated with a poor outcome. The abnormalities classified as high risk are:
 - iAMP21, t(17;19)(q22;p13), MLL (KMT2A) rearrangement, near haploidy and low hypodiploidy
- Good risk –the cytogenetic and chromosomal abnormalities classified as good risk, which are:
 - ETV6-RUNX1, high hyperdiploidy (51-65 chromosomes)

- Intermediate risk- all other patients were categorised as intermediate risk cytogenetics, including those with a missing or failed genetic analysis.

If a patient had high risk cytogenetics and high hyperdiploidy, the hyperdiploidy was secondary feature, and they were classified as high risk.

MRD status was categorised into low risk; intermediate risk; risk; or, no result.

Immunophenotype was categorised into B-ALL; T-ALL; B-LBL; or, T-LBL.

4.2.8.2 Radiology data

A central review panel consisting of Paediatric Radiologists with an interest in paediatric musculoskeletal imaging reviewed each MRI. The grade of ON was assessed using a modified scoring system using a study radiology *proforma* with additional descriptive analysis of MR imaging.

DXA and vertebral fracture assessment results were also reviewed centrally, with adjustments to bone mineral density using BMAD for the spine, and the height Z-score for TBLH [38]. The thoracic and lumbar vertebra were assessed (T4-L4 where possible), using the Genant semi-quantitative method [47].

4.2.8.3 Physiotherapy assessment

The physiotherapy assessments was processed as below:

The subjective assessment, the c-HAQ, was coded using the c-HAQ scoring system. The 8 categories within the c-HAQ are:

- Dressing and grooming
- Arising
- Eating
- Walking
- Hygiene
- Reach
- Grip
- Activities

Responses for each category were coded:

Without any difficulty=0

With some difficulty=1

With much difficulty=2

Unable to do=3

The highest score for any component question determined the score for that category. If a component question was left blank/ not applicable, the score for that category was determined by the remaining completed questions. If devices, aids or assistance were required for a category the minimum score was 2. If all components for a category were blank, then the category was not included.

The disability index was calculated by adding the scores for each of the categories and dividing by the number of categories answered, which gave a score between 0 and 3.

The disability index was supplemented with two visual analogue scales, one for pain and one for global assessment of overall well-being. Both were measured on a 0-10 scale, with 0 being no pain or no concerns, and 10 being severe pain or extremely bad overall well-being.

Where possible, elements of the objective physiotherapy assessment developed for this study were analysed as continuous variables. This included activity levels (hours), pain score (0-10) and power (0-5). Range of movement was not able to be analysed as a continuous variable as phrases such as 'end of range restriction' were used, and hence range of movement was coded as below:

Range of movement	Score
No restriction	0
End of range (<5 degrees restriction)	1
Moderate restriction (5-20 degrees restriction)	2
Significant restriction (>20 degrees restriction)	3

Qualitative statements, including gait analysis were analysed and categorised accordingly.

The final frozen dataset for the preliminary analysis was taken on 20/06/2019.

4.2.9 Data management and ethical permission

A Microsoft Access database recorded and linked all the socio-demographic and clinical data for a study participant with information from their radiology assessments. Data protection regulations (including EU General Data Protection Regulation 2016) at each centre were complied with.

The local clinical team will identify and provide age relevant patient information sheets to potential participants. Written patient consent or assent

will be obtained by the local clinical team, with parental consent obtained for patients under 16 years of age. Data was submitted centrally via a secure NHS email address with all patient identifiers removed. At each hospital site local clinicians and physiotherapists completed the relevant forms at each time-point, with forms anonymized locally prior to being returned to the central trial unit. Images of MRI and DXA scans were anonymised locally and placed onto CDs which were sent to the central trial unit.

Ethical approval for the study was granted by the Yorkshire and the Humber Sheffield research ethics committee on the 12th of July 2016. REC reference: 16/YH/0206 (Appendix 12)

A substantial amendment was submitted prior to initiation of the study at any sites on 12/03/2017, with a REC favourable opinion received on 12/04/2017. A further substantial amendment was submitted on 17/01/2018, with REC approval granted on 14/02/2018. Details of amendments are available in Appendix 13.

Trial registration number: NCT02598401

Date of registration: 05/11/2015

4.3 Results

4.3.1 Patient demographics

At time of data freezing, there were 22 potentially eligible patients. 19 of these patients consented for inclusion in the study. During the study, 3 patients were unable to continue to participate. 1 patient was unable to tolerate MRI scans, and 2 patients failed induction treatment. 18 patients were initially treated on regimen B, with 1 patient treated on regimen A at diagnosis. The patient who started on regimen A was unable to tolerate MRI scans, and withdrew from the study.

Patient demographics are presented in Table 29.

Table 29. British OsteoNEcrosis Study demographic and biochemical data

Demographic/ biochemical variable	Number of patients (% (where applicable))	Median (IQR)
Age	19	14.1 (12.0 to 15.3)
Ethnicity White British British Asian Black Other	14 (74%) 3 (16%) 0 2 (11%)	Not applicable
Sex Male Female	12 (63%) 7 (37%)	Not applicable
Multiple deprivation index	19	20475 (8135 to 27060)
BMI SDS	19	0.6 (-1.2 to 1.4)
Immunophenotype B-ALL T-ALL B-LBL T-LBL	12 4 0 3	Not applicable
Clinical features at diagnosis Duration of symptoms (months) Lymphadenopathy Hepatomegaly Splenomegaly Bone pain	19 8 (42%) 4 (21%) 4 (21%) 2 (11%)	1 (0.3 to 2.5) n/a n/a n/a n/a
Highest white cell count (x10 ⁹ cells/L)	19	13.6 (3.9 to 29.4)
Cytogenetics/ molecular status High Intermediate Good Not available	1 (5%) 10 (53%) 5 (26%) 3 (16%)	Not applicable
MRD status (end of induction) High risk Low risk No result	6 (32%) 7 (37%) 6 (32%)	Not applicable
Pubertal status Pre-pubertal In pubertal Completed puberty Not available	3 (16%) 4 (21%) 9 (47%) 3 (16%)	Not applicable
Treatment regimen (consolidation) A B C Off treatment	1 (5%) 8 (42%) 8 (42%) 2 (11%)	Not applicable
Biochemical results at diagnosis Albumin (g/L) HDL (mmol/L) LDL (mmol/L) Cholesterol (mmol/L) Triglycerides (mmol/L) PTH (pmol/L) Vitamin D (nmol/L) Alkaline phosphatase (U/L) Calcium (mmol/L) Phosphate (mmol/L)	19 16 16 13 15 13 14 16 16 16	35.0 (31.0 to 38.5) 1.5 (1.2 to 1.6) 2.5 (2.3 to 3.1) 4.8 (4.4 to 5) 1.4 (0.8 to 2.5) 5.1 (3.1 to 12.8) 45.0 (31.3 to 71.3) 111.5 (86.5 to 150.5) 2.2 (2.1 to 2.2) 1.3 (1.0 to 1.5)
Biochemical results at end of delayed intensification Albumin (g/L) HDL (mmol/L) LDL (mmol/L) Cholesterol (mmol/L) Triglycerides (mmol/L) PTH (pmol/L) Vitamin D (nmol/L) Alkaline phosphatase (U/L) Calcium (mmol/L) Phosphate (mmol/L)	9 8 8 8 7 6 8 9 7 7	40.0 (36.0 to 42.5) 1.1 (0.8 to 1.4) 2.6 (1.7 to 3.5) 5.0 (3.5 to 5.8) 1.2 (1.2 to 1.4) 4.6 (3.2 to 6.6) 52.3 (39.5 to 66.7) 97.0 (70.5 to 110.5) 2.4 (2.2 to 2.4) 1.6 (1.3 to 1.7)

4.3.2 MRI and physiotherapy results

At time of data freezing, MRI scan results were available for 18 patients, with 2 or more MRI scans available for 11 patients. Results are presented in Table 30.

The median time to first scan was 20 days after commencement of chemotherapy, (IQR 14.5-22 days). The median time to second scan was 233 days after initiation of chemotherapy (IQR 211-250 days).

Table 30. Magnetic resonance imaging and physiotherapy results

Patient number	Timing of MRI scan (days after start of treatment)	MRI right lower limb score	MRI left lower limb score	MRI descriptive comments	Hours of activity	Use of aids (specify)	Pain ^a (specify area and score)	c-HAQ Disability index	c-HAQ Overall pain General evaluation	Objective gait assessment	Objective joint movement assessment* (power, range)
1	20	0	0	Marrow oedema in distal femoral and proximal tibial metaphysis	2		0	1.00	Not completed	NAD	No limitation
	180	Femur 2 Tibia 2 Knee 3	Femur 2 Tibia 2 Knee 3	Classical ON, not in same areas as previous marrow lesions	0.5	Wheelchair (rarely)	Back 4 Knees 5	1.75	6 2	External rotation/ out-toeing	Hips (4) Knees (4) Shoulders (3,1)
	307 (extra scan- not BONES)	Femur 2 Tibia 2	Femur 2 Tibia 2 Knee 3	Classic ON lesions, prominent in distal tibial metaphysis. Marrow oedema in medial proximal tibia and distal femoral metaphysis							
	554	Femur 2 Tibia 2	Femur 2 Tibia 2	Classical ON. Marrow changes in distal femoral and proximal tibial metaphysis have resolved	1		Back 7 Hips 5 Knees 6 Ankles 6	0.88	6 7	NAD	Shoulders (4)
2	4	0	0	Marked marrow changes	10		0	0.00	3.5 3.5	NAD	Hips (4) Knees (4)
	223	Hip 4 Femur 2 Knee 4	Hip 4 Knee 4 Tibia 2	Classical ON	4		Back 5	0.5	3 0.5	NAD	Hips (4) Knees (4) Ankles (4)
	442 (extra scan of hips - not BONES)	Hip 5	Hip 5								
3	20	Femur 2 Tibia 2	Femur 2 Tibia 2	Diffuse abnormalities throughout the femoral and lower legs	4	Wheelchair Aids for getting up	Back 6***	0.89	0 0	NAD	Hips (4) Knees (4) Shoulders (4)

Patient number	Timing of MRI scan (days after start of treatment)	MRI right lower limb score	MRI left lower limb score	MRI descriptive comments	Hours of activity	Use of aids (specify)	Pain ^a (specify area and score)	c-HAQ Disability index	c-HAQ Overall pain General evaluation	Objective gait assessment	Objective joint movement assessment* (power, range)
	250	0	0		2	Wheelchair Bath rail	Back 7	1.63	6 2	Out-toeing	R hip (4) L hip (3,1) Knees (3) Ankles (4) Shoulders (4)
4	11	0	0	Diffuse abnormalities throughout all bones, no classical ON	7		0	0	0 0	NAD	No limitation
	200	Femur 2 Tibia 2 Fibula 2	Femur 2 Tibia 2 Fibula 2	Diffuse changes still present.	6.5		Back 3	0	0.5 0.3	NAD	Hips (4) Knees (4)
5	22	Femur 2	0	Marrow changes, especially over pelvis and proximal femur. One area of early ON in proximal femur	3		Back 5 Right hip 8 Left hip 6 Right knee 8 Left knee 6 Right ankle 6 Left ankle 4	0.38	8.7 8.5	Slight out-toeing	Hips (4) Knees (4)
	215	Femur 2	0	Classic ON in femur. Atypical patchy changes in pelvis	4		Shoulders 3 Back 5 Hips 4 Right knee 7	0.75	2.5 1	NAD	Hips (3, 1) Knees (4) Shoulders (4)
6	27	0	0	Diffuse marrow abnormalities	5		0	0	0 8	NAD	No limitation
	255	Hip 3 Femur 2 Knee 3 Tibia 2 Fibula 2 Ankle 2 Foot 2	Femur 2 Knee 4 Tibia 2 Fibula 2 Ankle 2 Foot 2	Classical ON	1.5	Wheelchair	Back 3 Knees 4	1.63	2 1.5	NAD	Right hip (4) Left hip (4,1)

Patient number	Timing of MRI scan (days after start of treatment)	MRI right lower limb score	MRI left lower limb score	MRI descriptive comments	Hours of activity	Use of aids (specify)	Pain ^a (specify area and score)	c-HAQ Disability index	c-HAQ Overall pain General evaluation	Objective gait assessment	Objective joint movement assessment* (power, range)
7	22	0	0	Small hip and knee effusions	6		0	0.5	0 3	NAD	No limitation
	310	Hip 3 Femur 2 Knee 4 Tibia 2 Fibula 2 Foot 2	Hip 4 Femur 2 Knee 4 Tibia 2 Fibula 2 Foot 2	Classical ON	2	Wheelchair	Shoulders 2 Back 8 Right hip 5 Left hip 4 Knees 6 Ankles 4	2.5	7.5 8	Wide base of support, short stride, flat foot strike	Right hip (4,1) Left hip (4) Knees (4) Ankles (4) Right shoulder (4,1) Left shoulder (4)
8	18	0	0	Small knee effusion	6		Back 6	0	0 0	Poor balance	Hips (4) Knees (4) Ankles (4) Shoulders (4)
	210	Femur 2 Knee 4 Tibia 2 Ankle 3 Foot 2	Femur 2 Knee 2 Tibia 2 Foot 2	Classical ON	2	Wheelchair, raised toilet seat	Back 6	1.75	3.5 3	Foot drop with peripheral neuropathy	Left hip (1) Knees (4) Ankles (3)** Shoulders (4)
9	30	0	0	Patchy changes throughout, no classical ON lesions	4		Back 8	0	0.1 0	Slight in-toeing	No limitation
10	110	0	0					1.75	0 4		No limitation
					5		Back 2 Right ankle 2	0.13	0.5 1	NAD	No limitation
11	21	0	0					1.71	Not completed	Antalgic gait	Knees (4)
12	28	0	0	Oedema right femoral neck				0.88	1 0.5	NAD	No limitation
13	11	0	0	Patchy oedema left femoral head and mid-tibia bilaterally. Small knee effusions	8			0.00	0 0	NAD	No limitation

Patient number	Timing of MRI scan (days after start of treatment)	MRI right lower limb score	MRI left lower limb score	MRI descriptive comments	Hours of activity	Use of aids (specify)	Pain ^a (specify area and score)	c-HAQ Disability index	c-HAQ Overall pain General evaluation	Objective gait assessment	Objective joint movement assessment* (power, range)
	248	Femur 2 Knee 3 Tibia 2 Foot 2	Femur 2 Knee 3 Tibia 2 Foot 2		1.5	Build up pencils/ special utensils	Back 3 Knees 3	2.00	3 Not completed	NAD	No limitation
14	20	0	0	Marked diffuse marrow change throughout, likely leukaemic infiltrate	4	Bath stool	Right knee 5 Left knee 5	1.13	4 4	Stiff	No limitation
	243	Pelvis 2 Femur 2 Tibia 2	Pelvis 2 Femur 2 Tibia 2	Classical ON lesions not involving joints	2		Shoulders 4 Back 7-10 Knees 5 Ankles 4	0.63	4 7	NAD	Right hip (1) Left knee (4) Right knee (4,1)
15	17	0	0	Marked diffuse marrow change throughout	3.5					NAD	No limitation
	236	Pelvis Grade 2, Femur Grade 2, Knee Grade 3, Tibia Grade 2, Fibula Grade 2, Ankle Grade 3, Foot Grade 3	Pelvis Grade 2, Femur Grade 2, Knee Grade 4, Tibia Grade 2, Fibula Grade 2, Ankle Grade 3, Foot Grade 3		2		Back 6	0.38	1 0	Slightly heavy gait	No limitation
16	21	0	0	Marked diffuse marrow change throughout	3.5		Right shoulder 3 Left shoulder 3	0.57	3 3	NAD	No limitation
17	12	0	0	Marked diffuse marrow change, soft tissue and muscle oedema	2		Right ankle 3	0.5	3 2.5	NAD	No limitation
18	28	0	0	Diffuse marrow changes	7			0	0 0	NAD	No limitation

For ON score: If all areas score the same, only one score is documented. If there is no specific score for an area, it has scored 0. NAD=no abnormality detected, ROM=range of movement.

^aFor pain score: Only areas with scores over 0 documented.

*For joint movement: if not specified, no limitation in power or range of movement. Power scored 1-5. R=right, L=left. ** Patient had full passive range of movement in ankles, but minimal active dorsiflexion due to marked peripheral neuropathy. *** Patient had pre-existing L5-S1 grade 1 spondylolisthesis

It can be seen that only two patients (Patients 3 and 5) had changes consistent with ON at the time of their first scan. These changes had resolved by the end of DI for Patient 3, but the patient continued to require the use of a wheelchair, possibly indicating that their functional limitations were unrelated to the osteonecrotic changes. Patient 5 had on-going changes in the femur, with persistent pain reported in hips and knees.

All other patients who had a second MRI scan developed at least one osteonecrotic area by the end of DI, with more than one area involved in the majority of patients. All patients who developed osteonecrotic areas by the end of DI had a reduction in self-reported hours of activity (median activity 4 hours at diagnosis (IQR: 3.5 to 6.5), which reduced to 2 hours (IQR: 1.9 to 4) by the end of DI). However, this was also the case for patient 3, whose osteonecrotic changes had resolved by the end of DI.

The most common aid used by patients was the wheelchair, used by 5 out of 10 patients by the end of DI.

The overall pain score increased from a median score of 0 (IQR: 0 to 3) at diagnosis of ALL (n=14), to 3.5 (IQR: 2.4 to 4.5) by the end of DI (n=12). The overall score of well-being stayed constant in patients, with a median score of 1.5 at the start of treatment and by the end of DI.

There were 3 patients who developed ON of one or more of their hips, (Patients 2, 6 and 7). Hip pain was reported in one of these patients, with limitation in power noted in 2 patients. Due to development of symptoms, Patient 2 went on to have a further scan at day 442, with progression of ON lesions to grade 5. Patient 1 also developed symptoms (knee pain) that clinicians felt were consistent with ON, and was found to have changes consistent with ON in femora, tibiae and one knee.

The c-HAQ disability index increased in 8 of 9 patients from induction to end of DI, with the median disability index increasing from 0.5 to 0.75 between the first and second time-point. Although all of the patients who had an increased disability index by time point 2 developed osteonecrotic changes during this period of time, the score also increased in the 2 patients who had ON at the first time point. Objective physiotherapy assessment found an increased limitation in power at the hips and/or knees in 9 of 10 patients who developed ON by the end of DI. Of the 18 patients assessed at baseline, 13 had no limitation in joint power or range (72%), which reduced to 4 out of 13 patients by the end of DI (31%). Areas that were found to have some degree of limitation at baseline were hips (reported in 22% of patients), knees

(22%), shoulders (11%), and ankles (6%). By the end of DI, some limitation was noted in 69% of hips, 62% of knees, 38% of shoulders and 31% of ankles.

4.3.3 Bone mineral density and vertebral fractures

DXA results were available for 16 patients who were recruited into BONES. Two patients had a second DXA assessment at time of data freezing. Initial BMD and VF assessment results are presented in Table 31, with BMD results graphically represented in Figures 29 and 30.

Table 31. Initial bone mineral density and vertebral fracture results

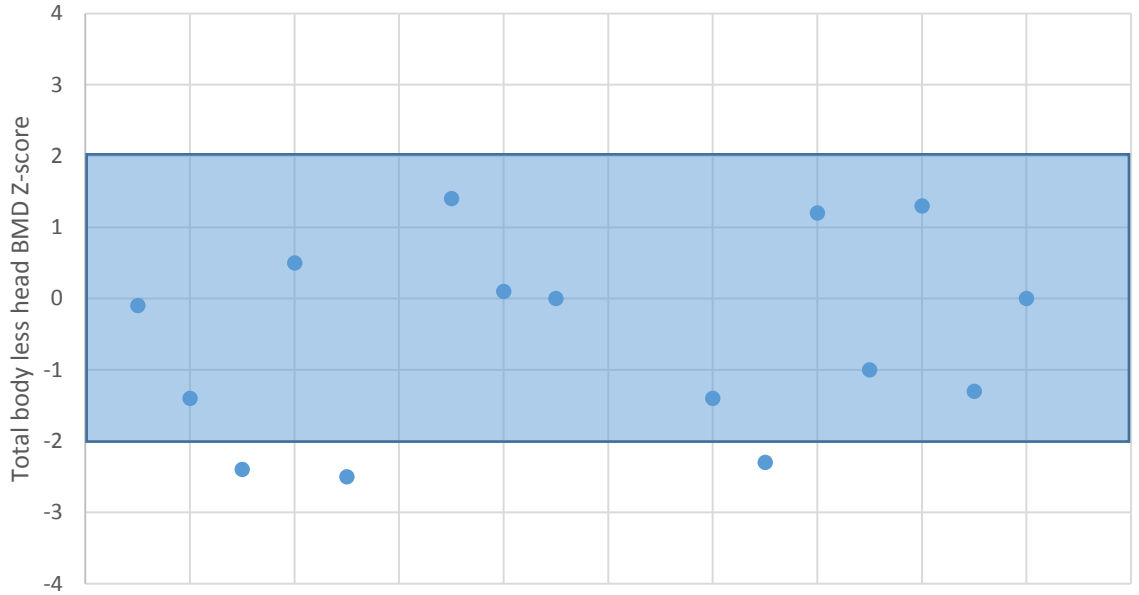
Patient number	Total body less head Z-score	Lumbar spine bone mineral apparent density Z-score	Vertebral fractures
1	-0.1	-1.5	0
2	-1.4	0.1	0
3	-2.4	-2.8	0
4	0.5	-0.4	0
5	-2.5	-2.7	0
7	1.4	3.5	0
8	0.1	-1.4	0
9	0	-3.3	Grade 1 T9 + T10
10	n/a	-1.1	n/a
12	-1.4	-0.7	n/a
13	-2.3	-1.9	0
14	1.2	1.9	Grade 1 L2, T5, T7
15	-1.0	-1.7	0
16	1.3	1.3	0
17	-1.3	-1	0
18	0	0.9	0

n/a: not available

T: thoracic vertebra

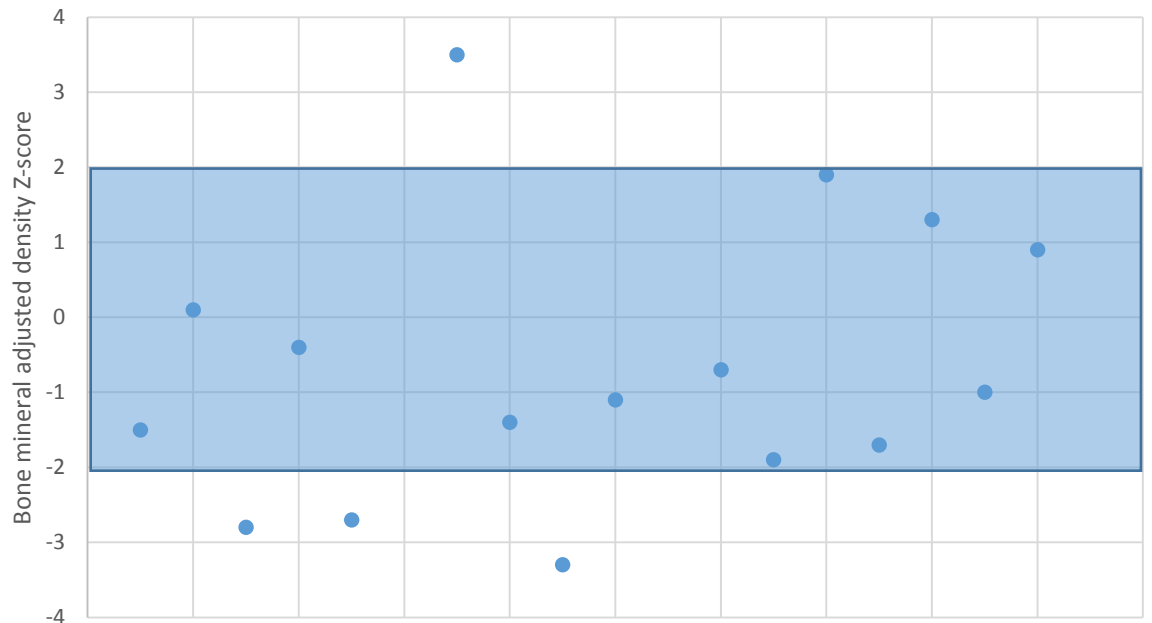
L: lumbar vertebra

Figure 29. Total body less head bone mineral density Z-scores at diagnosis of acute lymphoblastic leukaemia.



Shaded area represents normal range of TBLH BMD. Numerical value on x-axis equates to patient number.

Figure 30. Lumbar spine bone mineral apparent density Z-scores at diagnosis of acute lymphoblastic leukaemia



Shaded area represents normal range of BMAD Z-score. Numerical value on x-axis equates to patient number.

The median TBLH of this group of patients was -0.1 SDS (IQR: -1.4 to 0.3), with a median lumbar spine BMAD of -1.05 SDS (IQR: -1.75 to 0.3). Two out of fourteen (14%) of patients had evidence of VFs on their initial VF assessment.

Two patients (Patients 1 and 10) have had a second DXA assessment reported. For Patient 1, the TBLH Z-score and BMAD Z-scores reduced to -0.4 and -2.5 respectively. The lumbar spine BMAD for Patient 10 increased slightly to -0.8 SDS, with a TBLH of -1SDS. Neither patient had VFs identified.

Back pain was reported by a number of patients (Table 30), with an increase in back pain at the end of DI (median back pain score increased from 0 at ALL diagnosis to 3.5 at end of DI). Of the 2 patients with vertebral fractures at baseline, only one reported baseline back pain (Patient 9), although the other patient (Patient 14) had significant pain by the end of DI (pain score 8.5)

Chapter 5 Discussion

The central aim of this research was to gain a greater understanding of the bone health of children with ALL, with 2 main objectives to this work. The first objective was to complete a retrospective review of the cohort of patients enrolled in the national trial for children and young adults with ALL, UKALL 2003, which ran from 2003 to 2011. The primary aims were to:

- Report the UK prevalence of symptomatic ON in young people with ALL
- Describe the chronology of the development of symptoms related to ON and subsequent diagnosis of ON
- Identify risk factors for the development of ON
- Determine which joints are affected by ON and methods of diagnosis of ON in patients with ALL
- Describe the medical and surgical management of patients diagnosed with ON in UKALL 2003
- Establish the long-term outcomes of patients affected by ON in UKALL 2003

A secondary analysis of this population aimed to:

- Characterize the surgical procedures performed in patients affected by symptomatic ON in UKALL 2003, including the identification of sequential procedures in individuals.
- Evaluate the efficacy of femoral head core decompression in prevention of joint collapse in young people with symptomatic ON.

The second objective was to develop a protocol and establish a prospective longitudinal cohort study of young people with ALL or LBL which would aim to:

- Identify the incidence of symptomatic and asymptomatic ON in older children, teenagers and young adults being treated for ALL or LBL in the UK at different time points in their treatment
- Identify the risk factors for progression and the development of symptomatic ON in this population
- Identify specific radiological features which might predict for either progression or regression in those with asymptomatic ON
- Evaluate functional ability and explore the correlation of this with MRI findings

- Evaluate changes in BMD and VF incidence during treatment for ALL or LBL

Each objective will be reviewed in detail, with an evaluation of the strengths and limitations of the work.

5.1 A retrospective review of the UKALL 2003 cohort

5.1.1 UK prevalence of symptomatic osteonecrosis

The overall prevalence of symptomatic ON in the cohort of patients recruited to UKALL 2003 was 5.5% (n=170). This finding is consistent with other retrospective studies reporting upon the prevalence of symptomatic ON in patients with ALL, in which results ranged from 1-15% [109, 111, 112, 115, 123, 148, 149]. The figure is, unsurprisingly, much higher in prospective studies in which there is an assessment of asymptomatic osteonecrotic lesions [131, 132], or in studies evaluating only high risk patients [105, 119, 134].

There is a lower incidence of ON described in studies with no planned reporting of ON [116]. In UKALL 2003 there was reporting to the central trial unit of all patients who developed bone toxicity, which included ON, using toxicity reporting forms or serious adverse event forms. I found that whilst the central trial unit was informed of the majority of cases of ON, our longer period of follow-up and the use of a targeted questionnaire revealed an additional 55 patients, increasing the reported prevalence of ON in UKALL 2003 from 3.7% to 5.5% [128]. The central trial unit may not have been informed of the diagnosis of ON for a number of reasons, including late diagnosis of ON or a lack of awareness of reporting guidelines.

5.1.2 Chronology of development of symptoms and diagnosis of osteonecrosis

In UKALL 2003 symptoms of ON were reported at a median time of 14 months after the diagnosis of ALL, with ON subsequently diagnosed at a median of 16 months after diagnosis of ALL. The cumulative incidence of ON in all patients was 1.1% at 1 year, 4.0% at 2 years, 4.9% after 3 years, 5.1% at 5 years and 5.2% at 7 years. For patients over the age of 10 years at diagnosis of ALL, the cumulative incidence of ON was around 3 times

higher at all time-points: 3.3% at 1 year, 12.5% at 2 years, 15.1% at 3 years, 16% at 5 years and 16.2% at 7 years.

These results are similar to those previously reported in the literature, although there are few other retrospective studies with such detailed analysis of the timing of ON symptom development. Most previously reported studies also had much shorter periods of follow up, potentially missing cases of late onset ON. This study found 5% of patients were diagnosed with ON between 3 and 5 years after diagnosis of malignancy, with 1% of patients diagnosed with ON after 5 years. The longest time to diagnosis of ON was 6.26 years after the diagnosis of ALL [286]. Previously published retrospective studies suggest that the majority of patients who develop symptomatic ON do so within the first 3 years of treatment [109-111, 115, 119, 127, 150], with one of the most comprehensive studies reporting that 35% of cases occurred within the first 12 months after diagnosis of ALL [111]. Prospective studies suggest that asymptomatic lesions are likely to develop within the first year of treatment [132] with a study by the St Jude's group reporting that 38.7% of patients developed Grade 1 ON by 6.5 months after start of ALL treatment [132]. When patients were screened very early after the diagnosis of ALL (median 12.5 days), only 9.2% of patients were found to have any osteonecrotic lesions [320]. Therefore the critical time-point of lesion development remains unclear.

One difficulty with making a diagnosis of ON is the non-specific nature of symptoms. Patients undergoing treatment for ALL are exposed to a large number of chemotherapeutic agents, some of which have significant side-effects. Vincristine neuropathy is a well-recognised side effect of vincristine treatment [321] and can result in sensory, motor and autonomic neuropathy. Although the classical features of vincristine neuropathy are relatively distinct, there may be some overlap between pain due to ON and limb pain as a result of vincristine treatment, making a diagnosis of ON more challenging.

5.1.3 Risk factors for the development of symptomatic osteonecrosis

In this analysis age, ethnicity, sex and 1 versus 2 blocks of DI were assessed as possible risk factors for the development of ON.

After analysis, age was found to be the only risk factor for the development of ON, with an odds ratio of 22.96 (CI: 14.38 to 36.64) and 21.31 (CI: 12.09

to 37.57) for those aged 10-15 and 16-20 years respectively, when compared with patients aged <10 years. Patients over 20 years of age also had an increased risk of developing ON, with an odds ratio of 8.10 (CI 2.32 to 28.22) when compared with patients aged <10 years. This corresponds to the literature, which has universally found age >10 years at diagnosis of ALL to be a risk factor in the development of ON [109-112, 114-116, 119, 121, 127, 128, 132, 145]. There has been one previous study which reported adolescents younger than 20 years of age at diagnosis of ALL were at higher risk of developing ON, compared with older patients [123], and my results confirm this finding. The reason for this increased risk of ON in young people between 10 and 20 years of age is not fully understood. One hypothesis is that the increased risk of ON is related to puberty and concurrent increased height velocity, although this has not been proven.

My study did not find that the sex of the patient was a risk factor for the development of ON. This corresponds with the results of a number of other studies [110, 111, 114, 123, 125, 127, 131, 140, 170], although the literature is inconsistent in this area [100, 109, 117, 119, 134-136, 141, 144]. This is the only study to have used a causal inference model to justify the choice of confounders, and therefore the results are considered to be more robust than similar studies previously carried out.

One study found that the relationship between risk of developing ON and sex of the patient was dependent on the age of the patient [119]. In the study by Mattano in 2000 the gender difference was greatest in the 10-15 year age group, with 3 year rates of 19.2% for females and 9.8% for males [119]. However, among the smaller group of 16-20 year olds with ON, the ON incidence was higher in males than in females (20.7% v 13.2% respectively) [119]. An age by gender interaction for development of ON was not found in my study, which was considerably larger than those mentioned above, but it may be that the specific treatment regimen influences the importance of sex as a risk factor in the development of ON.

This study assessed the role of ethnicity on development of ON. However, categorisation of ethnicity poses many difficulties due to the subjective nature of identification of ethnicity [322, 323]. Comparison of ethnicity data with other international studies is challenging, as there are considerable inconsistencies between ethnic classifications amongst different countries, particularly with the term 'Asian'. In the UK, being of Asian ethnic origin typically refers to people from South Asian countries (predominantly India,

Pakistan and Bangladesh), whereas studies based in the USA typically used the term Asian for those of East Asian origin (Hans Chinese and Japanese ancestry). In the literature, a number of studies separated patients only into White and non-white, whilst others had only White, Black and Hispanic groups. Classifying patients as being of White race poses its own challenges, as the term may be considered to be a social construct, which does not incorporate the reality of biological variation between different populations. Few previously reported studies commented on Asian patients at all and most studies where race was commented upon were composed of predominantly White patients. Some studies have reported White race to be a risk factor for development of ON [117, 119, 146], but this has not been a consistent finding [131, 132].

In this study of patients recruited into UKALL 2003, no single ethnicity was found to be a risk factor for the development of ON. However, the risk of ON in the Asian population compared to the White population neared significance ($p=0.053$, 95% CI 0.69-2.07). As Asian patients were only 2% of all trial patients, these results suggest that with larger numbers of Asian patients, statistical significance would have been achieved. That ethnicity could have an impact on incidence of ON is biologically plausible, due to the differences in bone size, shape and density in people of different ethnic backgrounds [324, 325], but the mechanism is unclear, particularly given that the development of ON was not found to be associated with BMD at baseline [130]. It may be that different ethnic groups have variances in genetic risk factors, putting them at differing levels of risk depending on their specific treatment regime.

No relationship was found between the number of blocks of DI and prevalence of ON. As an additional block of DI would result in an additional 2 weeks of dexamethasone at a dose of $10\text{mg}/\text{m}^2/\text{day}$, it might have been anticipated that this would have resulted in an increased risk of ON development. The lack of increase in reported cases of ON may be due to the timing of the additional dexamethasone, which was at week 32 and 34 of treatment. Studies suggest that osteonecrotic lesions are likely to have already developed by week 32 [132]. Consequently, dexamethasone given later in the treatment course may be less influential in the development of ON.

5.1.4 Joints affected by symptomatic osteonecrosis and methods of diagnosis

This study found that the majority (85%) of patients in UKALL 2003 who developed ON had multiple affected joints. The joints most commonly affected by ON were hips and knees (34% and 32% respectively), followed by shoulders and ankle joints (14% and 10% respectively). The hip joint was affected in 58% of all patients with ON, with 2 hips affected in 35% of patients. At least one hip or knee was affected in 89% of patients. Previous studies also report that the greatest burden of symptomatic ON is in the lower limbs. One retrospective study reported that 46% of patients affected by symptomatic ON had at least one hip affected, with hip(s) and/ or knees affected in 85% of patients [119]. My study also found that 24% of all patients affected by ON had one or more shoulder joint affected, and this high prevalence of ON in shoulder joints has not been previously reported.

5.1.5 Methods of diagnosis of osteonecrosis

As would be expected, the majority (82%) of patients in UKALL 2003 had the diagnosis of ON confirmed by MRI, although there were centre specific differences, with plain X-rays used for diagnosis in 16% of patients. MRI is the most sensitive modality for detecting and diagnosing low grade ON, with plain radiographs only able to detect more advanced disease [159, 161, 164]. MRI also allows quantification of the area and extent of ON [292], and is generally the imaging modality of choice, particularly in the early stages of ON.

5.1.6 Medical management of patients diagnosed with osteonecrosis

The results from this study highlight the significant national variation in management of patients with ON. This was likely to be due to both lack of an evidence base for the management of patients, and lack of national consensus guidance. There were clear regional preferences in certain practices. For example, the decision to stop steroids after diagnosis of ON would appear to be centre dependent. There is no literature to support or reject the practice of cessation of steroids during the treatment of ALL as a means of preventing further deterioration of ON lesions. As lesions are likely to develop significantly earlier than the onset of symptoms [132], the cessation of steroids at the point of development of symptoms may be hypothesised to be too late, whilst potentially worsening mortality from

inadequate management of the underlying leukaemic process. The counterargument is that continuation of steroids could stimulate progression of existing osteonecrotic lesions, and allow development of additional lesions, particularly given that patients who develop ON generally develop multiple lesions affecting numerous joints. Without further research there can be no recommendations regarding best practice in this area.

Bisphosphonates were used in a number of centres, with pamidronate the agent most commonly used. Some form of bisphosphonate was given to 27% of patients (n=43), with use of bisphosphonates potentially unrelated to low BMD in 84% of cases. The review of use of bisphosphonates presented in Chapter 2.3.1 suggests that some bisphosphonates (namely pamidronate and zoledronate) may be beneficial for pain management [234, 241-243], with a number of studies also reporting improvement in functional ability in patients with ON who are treated with bisphosphonates [234, 241, 244, 245]. There is no evidence that bisphosphonates alter the radiological progression of ON in young people [241, 243, 245], although the literature is limited. Studies suggest that bisphosphonates are generally well tolerated, with minimal short-term side effects other than an initial acute phase reaction [326]. However, given the poor quality of the existing literature, additional research into the long term side effects and the specific use of bisphosphonates in young people with ALL or LBL and ON may be warranted.

Given the role of vitamin D in the maintenance of bone health, it may be expected that vitamin D supplementation would be prescribed to individuals with vitamin D deficiency and ALL. In our study vitamin D supplementation was provided to patients in 32% of cases; 36% received no such supplementation and provision was unclear in the remaining 32% of cases. However, vitamin D levels and timing of treatment was not requested, and specific conclusions cannot be drawn from these data. In the systematic review described in Chapter 2.3.2 there were no reported studies which assessed the impact of treatment of vitamin D deficiency on prevalence of ON. Within some areas of the paediatric haematology community there has been a reluctance to start vitamin D supplementation during induction therapy, due to the *in vitro* study by Antony et al [281]. As discussed in chapter 2, delaying treatment purely on the basis of the results of this study is not appropriate, and our systematic review found that in young patients with ALL, cholecalciferol has a good safety profile. However, there are

concerns that apparently innocuous medication, including bisphosphonates and vitamin D, may have unintended negative consequences on cure rates if appropriate research has not been conducted in the patient population of interest. This is of particular importance if medication is being given for potentially limited benefit.

5.1.7 Surgical management of patients diagnosed with osteonecrosis

Overall, the high rates of surgical intervention is one of the most striking outcomes of this work. In the initial analysis of results, of the 170 patients who were diagnosed with ON, 38% were reported to have had surgery related to their ON (n=65), and 19% of patients were reported to have at least one hip replacement. However, these numbers rose in the detailed analysis of surgical interventions performed, with 26% of patients reported to have at least one hip replacement, and some form of surgical intervention being performed in 47% of patients. In this second analysis hip replacements were performed in 43% of all hips affected by ON and arthroplasty was carried out in 21% of all joints affected by ON. Core decompressions were performed prior to replacement in 31% of patients who went on to have a hip replacement. In comparison, a retrospective study in the USA found that 22.7% of patients who were diagnosed with ON had at least one surgical procedure performed, with only 6% of patients requiring joint replacement [115]. However this study had a much shorter follow-up period (5 years from diagnosis of ALL), which is likely to result in an underestimation of the number of patients having surgical interventions. Our results for hip arthroplasty are similar to those of patients recruited to NOPHO ALL2008, where of the 65 patients with ON, 15 of 33 patients with grade 4 ON had arthroplasty performed (45%), with bilateral hip replacements in 9 patients (27%) [120]. In that study of patients, only 1 patient underwent core decompression, highlighting the differences in patient management internationally.

It can be seen that in our second analysis there was a higher rate of surgery reported compared with our earlier work. This may be due to the longer duration of follow-up, together with an altered emphasis in data collection. By collection of orthopaedic letters and operation notes we were able to obtain detailed information on all procedures performed, with confirmation of timing and surgical techniques used. When the results from the two studies are compared (Figures 31 and 32), it can be seen that whilst the numbers of

hips and shoulders affected by ON were appropriately represented in the second survey, the numbers of knees and ankles were underrepresented. This may be because knees and ankles are less likely to be referred to orthopaedic surgeons due to limited surgical options, or because symptoms are more likely to spontaneously resolve. In our analysis of patients with ON affecting knees and ankles, very few had significant surgical intervention. Figure 32 illustrates the much higher rate of arthroplasty in our follow-up analysis, which is likely to be due to the reasons detailed above.

Figure 31. Joints affected by osteonecrosis: a comparison of patients with confirmed osteonecrosis and those for whom second questionnaire responses were received

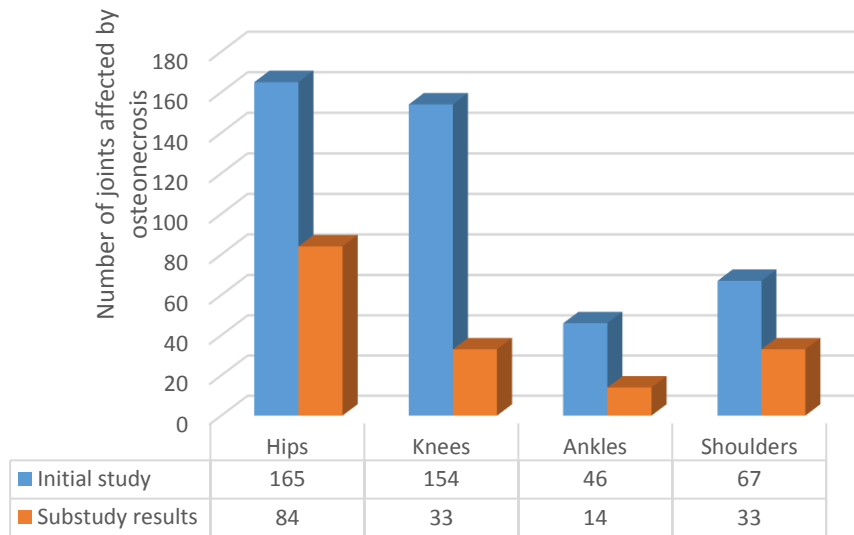
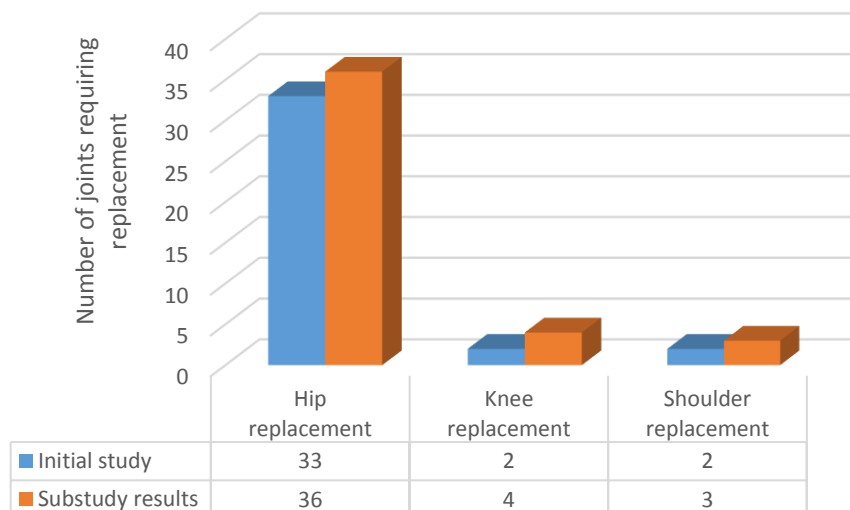


Figure 32. Joints replacements due to osteonecrosis in UKALL 2003: a comparison of patients with confirmed osteonecrosis and those for whom second questionnaire responses were received



When timing of surgical intervention was assessed, it was found that the median time for core decompression was 20 months after diagnosis of ALL. This would typically be whilst the patient is still receiving ALL treatment. In contrast, joints were replaced at a median time of 3.83 years after ALL diagnosis, suggesting that surgeons would usually wait until completion of chemotherapy before undertaking arthroplasty.

Our study also highlights the variation in surgical techniques used across the UK. A variety of prosthetic materials were used for joint replacements, with differences in fixation techniques. At present the optimal technique for hip replacement in this population is unknown. One study reported that at 10-year follow up there were lower rates of loosening in hips that had cementless total hip replacements than in hips that had cemented replacements [327]. However the mean patient age in this study was 43.3 years, with a mean BMI of 30.6kg/m². The study assessed patients with a range of different underlying pathologies, which could independently influence rate of loosening, as conditions such as Gaucher disease, sickle cell disease and renal failure are considered high risk conditions [328]. Much longer follow-up data would be of value to determine how this distinct patient population should be managed if joint replacement is required.

Core decompression was performed in 30% of hips affected by ON. Core decompression as a procedure for patients with ON was first introduced in the early 1960's, when Arlet and Ficat proposed to investigate osteonecrosis by a 'forage-biopsie' [156]. They introduced the concept of a core biopsy [329], which was later popularised by Hungerford as femoral head core decompression [330]. Core decompression of the femoral head involves drilling a hole through the distal aspect of the greater trochanter. The proposed mechanism of action of core decompression includes a direct reduction in intramedullary pressure and induction of limited tissue damage to promote healing, including vascular sprouting and angiogenesis. There are modified versions of this technique, with incorporation of grafts and injection of bone morphogenic protein, or autologous bone marrow. At present there is no agreement on the technique that will give the best results. In our study, 25% of patients had Osteoset® bone graft substitute used, and the most common technique was using 2 cannulated drill passes, but this ranged from 1 to 5 drill passes. A comparison of traditional core decompression and multiple drilling in patients with sickle cell disease found that at a mean follow-up of 3 years, there was no difference in the odds of

improvement by procedure [331]. Studies of adjunctive therapies with decompression are restricted by sample size and quality of evidence, and there is no current recommendation for use of any adjuvant therapy [332].

5.1.8 Efficacy of core decompression in prevention of joint collapse

This is the first study to compare femoral head core decompression with no joint preserving surgical intervention for the management of ON of the femoral head in young patients with ALL. The survival analysis found there to be no significant difference in femoral head survival (joint collapse or THR) between those patients who had core decompression compared with conservative management, although the hazard ratio suggested that core decompression was associated with a 20% lower risk of joint failure compared with conservative treatment. Therefore, it is possible that with a larger patient group significance would have been achieved. The lack of significance has potentially important implications for patient management, as core decompression was performed in 30% of hips affected by ON in this specific patient population, and highlights the value of a larger study of this subject.

It was found that the majority (85%) of patients had grade 4 or 5 ON at diagnosis, and our results cannot be extrapolated to individuals who are diagnosed with ON at grades less than 4. These results may suggest that in the UK ON in young people with ALL is diagnosed too late for effective surgical interventions to prevent hip collapse.

Previous studies assessing efficacy of core decompression in preservation of the femoral head have shown varying results, with grade of ON at time of intervention likely to be of critical importance. One of the earliest prospective studies was conducted in 1974 by Fairbank et al [333] and assessed 90 adult patients with 128 affected hips. The total failure rate (conversion to hip replacement) was 43%, with a higher failure rate in those who had a higher stage of ON at the time of core decompression (73% compared with 22%) [333]. There have been 2 randomised studies comparing core decompression with conservative management. A study conducted in the USA by Stulberg et al [334] randomised 55 hips in 36 adult patients to core decompression or conservative treatment. They reported that core decompression produced better clinical results than conservative treatment in the early stages of ON, but less successful results were seen in when osteonecrosis had progressed further. However, the need for further surgical

intervention was used as the end-point, which may be affected by other considerations, such as patient choice/ insurance coverage. A study reported by Koo et al [335] randomised 33 patients (37 hips) with early ON without radiological evidence of collapse to core decompression with cancellous bone graft, or conservative management. The primary end point was collapse of the femoral head. Survival analysis showed no significant difference in time to collapse between the two groups and by 24 months, 72% of hips in the core decompression group and 68% of hips in the non-operated group had undergone THR ($p=0.8$). In both of these randomised studies some patients had more than one affected hip randomised into the study with no adjustment in analysis used, potentially violating the independence of failure times assumption required in survival analysis. One study which included paediatric patients (age >10 years) was a randomised study of 38 patients with sickle cell disease and Steinberg stage 1-3 ON. Patients were randomised to core decompression and physiotherapy, or physiotherapy alone. This study found that physiotherapy alone appeared to be as effective as core decompression in improving hip function and survival [336]. A meta-analysis published in 2016 assessed the role of core decompression compared with all other joint preserving treatments in delaying the development of hip osteoarthritis [337]. Outcomes evaluated were patient clinical status, radiographic progression and need for total hip arthroplasty or further surgery. With a total of 12 studies, 5 of which were randomised trials, there was slight superiority of other joint preserving therapies compared with core decompression for all of the outcome measures. The main issue with this meta-analysis was the significant heterogeneity between studies, and the variation in the management of the control group.

5.1.9 Long-term outcomes of patients affected by osteonecrosis

This is one of the few studies to specifically assess the long term effects of ON on young people treated for ALL. At a median follow up time of 70.5 months after diagnosis of ALL, 39% of patients who had ON were reported to have no long term effects, with 38% reported to have minimal disability. Significant disability was reported in 9% of patients, with 3% of patients reported as requiring a wheelchair.

In the follow-up study of these patients, at a median of 83 months, 60% were reported to have returned to full mobility, and 71% of patients were reported to have either no pain, or only occasional pain.

It was reassuring to find that despite the often debilitating effects of ON in the short term, long term outcomes are good for the majority of patients. However, it must be borne in mind that these encouraging results are often after surgical intervention, and patients may need further revision surgeries in the future. A modern artificial hip joint is designed to last for at least 15 years, with a recent meta-analysis suggesting that a hip replacement can now be expected to last around 25 years in 58% of patients [338]. However, hip replacements in young patients have higher rates of revision surgery than in older patients [339, 340] and our cohort of patients are very likely to require further surgical intervention and revision surgery.

5.1.10 Strengths and limitations

This is the largest single UK study reporting symptomatic ON in children, teenagers and young adults with ALL providing long term follow up data of patients. Strengths of this work include the use of a national, high quality dataset with confirmed diagnosis of ALL and ON, the high questionnaire response rate (90%) and the extended follow-up period (median: 8.9 years). This study provides a more comprehensive assessment of patient demography and management than previous studies assessing patients with ALL developing ON, as well as identifying patients who were not previously reported to have ON. The subset analysis of surgical management of patients is the largest assessment of its type, and is the first time that survival analysis of hips affected by ON in young people with ALL has been reported, comparing core decompression with conservative management.

As described in the methodology, the minimal sufficient sets of confounders for adjustment was based on our development of a DAG. Chapter 3.2.6 describes the use of DAGs as a mathematically rigorous method for minimising bias and determining true confounders [284]. However, it is also important to recognise the limitations of DAGs. One of the main limitations is that determination of confounders is based upon the premise that the background information provided by the person developing the diagram is correct. Some of the assumptions made are of unknown validity and some are untested. The graphical assumptions are qualitative and nonparametric, and imply nothing about the specific functional form of the relations or distributions amongst the variables. However, these limitations are largely outweighed by adopting a robust, causal inference methodology which is widely accepted as the optimal statistical approach in identifying true confounders, such that model parsimony is respected [284].

One of the main limitations of this work is the retrospective nature of the study. This may result in recall bias, with more severe forms of ON potentially recorded or recalled. This may have enriched our data with a higher percentage of adverse outcomes, with patients who have had surgical procedures more likely to be recollected.

During questionnaire development a balance had to be made between depth of information collected and ease of questionnaire completion for responders. There are a number of sections in which additional detail would have been of value, particularly in the areas questioning medical management and long term outcomes. In retrospect, the question regarding the use of pamidronate would have elicited more information if it was changed to include the use of bisphosphonates more generally, with a request for information regarding indication, dose and duration of use. When identifying patients for whom steroids were stopped, it would have been of benefit to establish the date when steroids were stopped, timing of cessation of steroids and rationale for cessation. More information around the use of vitamin D would also have been of benefit, with identification of vitamin D deficient patients, PTH levels, treatment used and timing of treatment. Expansion of these sections would have enabled us to gain a much greater understanding of management decisions made.

Depending on the local situation, the questionnaire was variously completed by data managers, research nurses, administrative assistants or clinicians. This could potentially lead to inconsistency in the manner of completion. However, through regular correspondence with each centre, it was clear that data managers completed the vast majority of questionnaires, which is likely to minimise potential variation. In this study the method of determining ethnicity was not clearly defined, largely relying upon data inputted at admission to hospital. Patients and families are not consistently requested to describe their ethnicity on admission to hospital, which could result in incorrect assumptions being made.

In UKALL 2003 patients were not prospectively imaged for assessment of asymptomatic ON, and there were no specified thresholds for imaging of patients or criteria for joint imaging. In the initial study MR images were not centrally reviewed, and local reports were used to determine the diagnosis of ON. Therefore, the first part of this study did not incorporate grading and severity of ON, due to variability in MRI reporting across centres. Although

MR images were requested for the additional analysis, we were only able to obtain information for 50% of patients identified.

The outcome data collected by this study were limited due to a lack of well recognised specific, objective, measurable outcome measures for this patient population. Long term effects of ON were defined as the effect of ON at the most recent follow-up consultation. This varies significantly depending on whether the patient had or was due surgery, and our results were dependent on the level of documentation in the notes and clinic letters. Some of the outcomes, such as pain or mobility status may also be affected by factors unrelated to ON. It was also not possible to separate outcome data for different interventions (e.g. core decompression), as in many cases there was variation in the management of different osteonecrotic lesions within one individual. Questionnaires completed by the patients themselves could improve the quality of the data in this area, ideally with a control group of patients who received treatment for ALL but were unaffected by ON.

There are a number of limitations to the survival analysis of hips affected by ON. Of all the patients identified as having ON affecting the femoral head in UKALL 2003, imaging at diagnosis was available for only 36%. However, when patient demographics were analysed, there appeared to be no differences between those who were identified in the first round of data collection and those for whom results were available for the subsequent detailed analysis. As mentioned, in this study patients were not routinely imaged during ALL treatment, and the majority (85%) of patients had grade 4 or 5 ON at first imaging. In a survival analysis the 'ideal' starting point would be to measure the survival time from the moment the participant developed ON, but without prospective imaging this is not possible. The lack of routine imaging after diagnosis of ON also meant that it was not possible to determine accurately the true time to event (development of grade 5 ON), which would improve the accuracy of results. It may be that core decompression has a beneficial impact on pain, but this could not be assessed in this study, due to lack of standardised collection of this information. Variation in the care of patients across the country may also have affected the results. Differences in surgical techniques were described earlier, and it is possible that there was also variation in the conservative management of patients.

5.2 Development of a protocol to establish a prospective longitudinal cohort of young people with acute lymphoblastic leukaemia of lymphoblastic lymphoma

BONES is the first multi-centre prospective study using MRI imaging for the assessment of asymptomatic ON in the UK. It is also the first study to combine physiotherapy assessment with imaging and biochemical results.

5.2.1 Challenges

The development of BONES has been discussed at length in Chapter 4, with a discussion of the rationale for study design. A collaborative approach to study design was extremely beneficial in ensuring that the study had a robust methodology, but the co-ordination of a large multi-disciplinary team was time-consuming and at times challenging. The consultations conducted as part of the methodology development process revealed significant variation in practices across the country, particularly with respect to physiotherapy input. These needed to be accommodated within the study design, with provision for centres with more limited physiotherapy involvement.

The other major obstacle was a lack of capacity in interested centres. The consultations conducted as part of the scoping work confirmed that there was widespread interest in gaining further information about the natural history of osteonecrotic lesions in this patient population. Despite this enthusiasm and understanding, there were a number of issues that impeded the opening of the study in additional centres. One of the main limitations was a perceived lack of availability of paediatric MRI slots. This restriction prevented at least 2 centres from opening, despite interest from local clinical and research staff. In our own centre, limited MRI capacity delayed the start of opening of the study by 5 months. Another factor that limited the opening of the study in a number of centres was the lack of NIHR portfolio status. Unfortunately portfolio status was declined as the funding for the study was from a local charity. This meant 3 interested centres were unable to participate. Had we appreciated this impact, funding from a national body would have been pursued.

5.2.2 Analysis of preliminary results

These preliminary results need to be treated with caution due to the small number of patients with results available at time of data freezing.

It was found that only 2 of 18 patients (11%) had osteonecrotic changes at the time of their first MRI scan. This had risen to 10 out of 11 patients (91%) having osteonecrotic lesions by the end of DI. These results suggest that the majority of patients develop ON between the end of induction of chemotherapy (median time of first scan was 20 days), and prior to starting maintenance therapy. This is consistent with other prospective studies, where osteonecrotic changes were rarely seen at diagnosis [133], but were noted in 41% of patients at 6.5 months after initiation of treatment [132]. Given our patient population (patients aged 10-25 years), a higher rate of ON compared with previous studies is to be expected, although a rate of 91% by end of DI was not anticipated. In the 2 patients who had osteonecrotic changes at diagnosis, lesions had resolved by the second imaging point in one patient. This may have important implications for management of patients, as it may be possible to initiate prophylactic therapy after the intense induction period of chemotherapy has been completed.

As we continue to follow up this patient group, we will be able to understand more about how lesions evolve, and risk factors for development of symptomatic ON. Although osteonecrotic changes were seen in most patients by the end of DI, the majority of changes were in the diaphyses of the bones, with ON affecting the hips in only 3 out of 11 patients, and knees in 7 patients. It has been described that ON affecting the articular surface results in a poorer prognosis [159, 165-167], and the clinical impact of ON affecting the femoral head has been described in detail [169, 286]. Two of the patients with ON affecting a joint became symptomatic and had non-study MRI scans. One patient with symptomatic ON had a non-study scan undertaken, at day 442. ON had progressed from grade 4 at day 223 to grade 5 (joint collapse) by day 442. This highlights how lesions may rapidly progress, with subjective patient symptoms potentially only described with advanced stage lesions.

By the end of DI there was an overall increase in the disability index and pain scores of patients. At present, it is not possible to correlate these results with the development of osteonecrotic lesions, but it is hoped with more patients and a longer duration of follow-up we will be able to establish presence or absence of correlation. One of the difficulties in the assessment of patients with ON is that patients may have a number of co-morbidities. Vincristine causes axonopathy that manifests as a slowly progressive axonal

sensorimotor neuropathy [321]. Vincristine associated peripheral neuropathy is experienced by nearly all children who receive vincristine treatment [341], although the severity varies. In most cases the neuropathy progresses from distal to proximal regions, typically affecting lower limbs first. In the first year of vincristine therapy, hyporeflexia is the most common manifestation, followed by decreased sensation and strength [341]. The physiotherapy assessments carried out as part of this study are not specific enough to only detect limitations related to ON. Limitation of gait, power or range of movement could reflect vincristine related neuropathy, although it would be expected that the pattern of findings would be different to those of a patient with limitation due to ON.

After analysis of DXA results, it was found that at diagnosis the median TBLH of this group of patients was -0.1 SDS (IQR: -1.4 to 0.3) with a median BMAD of -1.05 SDS (IQR: -1.75 to 0.3). Other studies have reported that BMD at diagnosis of ALL, when measured by DXA, was lower than that of healthy controls [41, 130, 203], and report similar results to the median BMAD SDS presented in our data.

It can be seen that only 2 of 14 patients (14%) had evidence of vertebral fractures on their initial vertebral fracture assessment. This is similar to the findings by Halton et al [215], who found that in vertebral radiographs taken within 30 days of chemotherapy initiation, 16% of patients had prevalent vertebral fractures. Of the 2 patients with vertebral fractures in our study, fractures were grade 1 in both patients. Fractures affected 2 vertebrae in one patient, and 3 vertebrae in the other. In the study by Halton et al, 52% had one prevalent vertebral fracture, 27% had 2 to 5 fractures and 21% had between 6 and 10 fractures, with grade 1 fractures in 48% of patients [196].

Additional results will add to the robustness of our dataset, and it is hoped that subsequent scans will enable us to identify risk factors for development of vertebral fractures.

5.2.3 Future data management plan

Following completion of this thesis, the data will be stored on a secure network area within the University of Leeds, School of Medicine. Access will only be available to N. Amin, R. Feltbower and S. Kinsey. A request for funding for a research nurse has been made, to allow ongoing data collection and analysis.

5.2.4 Strengths and limitations

The main strengths of BONES are the prospective nature of the study and the multidisciplinary team involved in study design, which included paediatric radiologists, endocrinologists, orthopaedic surgeons and physiotherapists. By using the expertise and knowledge of a diverse group of experts we have been able to develop a study that has brought together a range of different elements in the assessment of each patient.

One of the main limitations to the BONES study is the small sample size. This is due to practical and resource constraints. However, despite the small sample size, the data collected provides a holistic approach to patient assessment that has not previously been undertaken.

During the analysis of patients in UKALL 2003 with symptomatic ON the striking variation in patient management was apparent. This lack of standardisation of care of patients, which reflects the lack of an evidence base for the management of patients with ON, also affects patients in the BONES study. Bisphosphonates have been started in one patient and vitamin D therapy in another, which may modify our findings.

The physiotherapy assessment, with use of both objective and subjective patient assessment, is one of the unique elements of this study. This will enable us to understand patient perception of pain and disability, together with standard methods of objective patient assessment. However, the physiotherapy assessment is limited by the lack of a validated scoring system for assessment of ON in patient with ALL. The discriminatory ability of the c-HAQ and the objective assessment was unclear at the start of the study. Whilst the c-HAQ was chosen as one of the most widely used measures of functional health status of children with musculoskeletal difficulties, it was noted that c-HAQ suffers from a ceiling effect in juvenile idiopathic arthritis. This means it is impossible to measure improvements at the better end of the functional spectrum, with a concurrent reduction in clinical validity [315, 342]. Revised versions have been developed to try to enhance discriminative validity, but results have been inconsistent [315]. The addition of more challenging items in a revised version of the c-HAQ reduced the ceiling effect in patients with juvenile arthritis a number of studies [315] but other revisions were less able to distinguish between patients and controls. It may be that revised or alternative methods of assessing subjective functional status of patients with ON would be

preferable, and this is something that would be of significant value to develop in further studies with larger cohorts.

When assessing preliminary study results it was clear that back pain was common in patients enrolled in the study. In retrospect formal objective assessment of the back would have been of benefit and should be included in future studies.

This is an observational study that aims to obtain detailed information about the development of ON in patients being treated for ALL or LBL. One aim of this research was to help understand some of the complex relationships between multiple different variables. However, correlation is not causation, and it may be that it would have been desirable to measure other unassessed variables, such as blood pressure or markers of infection. It is also possible that patients who are involved in the study may be more aware of ON, resulting in heightened concerns about pain or limitation in movement.

Chapter 6 Conclusion

6.1 Main findings

The first part of my work reported upon the prevalence and risk factors for the development of symptomatic ON in young people with ALL who were recruited into UKALL 2003. I then went on to describe the chronology, risk factors and management of ON in this population. This is the first time this has been conducted in such detail and a number of novel findings have been reported.

The overall prevalence of symptomatic ON in the cohort of patients recruited to UKALL 2003 was found to be 5.5%. As has previously been reported, age was the main risk factor for development of ON, and in this study 18% of patients aged between 10 and 20 years at diagnosis of ALL developed ON.

Symptoms of ON were most commonly reported in lower limbs, with multiple joints affected in the majority of patients. One of the most significant findings to emerge was the high rate of surgical intervention in patients. Hip replacements were performed in 43% of all hips affected by ON, with core decompression performed prior to replacement in 31% of affected hips. This is the first study to specifically assess the impact of core decompression in hips affected by ON. Survival analysis found no significant difference in femoral head survival (development of grade 5 ON or THR) between patients who had core decompression compared with patients who were managed conservatively; although with larger numbers significance may have been established. This is the first UK study to use the Niinimäki classification to assess the grade of ON. It was found that the majority of osteonecrotic lesions affecting the femoral head were grade 4 or 5 at diagnosis. This is an important finding as the late stage of ON at diagnosis may impact upon the efficacy of any interventional therapy.

This thesis also describes the establishment of a prospective longitudinal cohort study of patients aged 10 to 25 years with newly diagnosed ALL or LBL. This study assesses the prevalence and development of symptomatic and asymptomatic osteonecrotic lesions, together with BMD and VF incidence in patients with ALL or LBL. The main findings to date are that osteonecrotic changes typically occur between the end of induction and the start of maintenance therapy. The majority of lesions affect the diaphysis of the bones, but lesions affecting the articular surface may rapidly progress,

with symptoms only recognised after joint collapse. Vertebral fractures were reported in 14% of patients around the time of diagnosis of ALL.

Additional results will provide us with more robust data, and it is hoped that these will inform future studies and improve patient care.

6.2 Clinical implications

These results describe the high prevalence of ON development during treatment for ALL, particularly in young people who are aged 10-20 years at diagnosis of ALL. They also describe the high rates of surgical intervention in affected patients. This work can help UK clinicians provide accurate prognostic information about ON to patients with a diagnosis of ALL. ON should be discussed with patients at greatest risk (those aged 10 to 20 at diagnosis of ALL), and patients in the UK can now be provided with an age specific risk of ON development. In patients with symptomatic ON previously unavailable joint specific prognostic information and long term outcome data can now be offered.

An important finding of this work is that the diagnosis of symptomatic ON in patients with ALL typically occurs when lesions are grade 4 or 5, using the Niinimäki classification for ON. No significant difference was found between patients who had core decompression, compared with conservative management, in prevention of hip collapse in patients with ON affecting the femoral head. However, the hazard ratios suggest that with much larger numbers of patients, significance may have been achieved, and a prospective multicentre study may be of value. This is clinically important as unnecessary surgical interventions can put patients at risk from both surgical complications and the risk of anaesthesia.

6.3 Ongoing and future work

It is hoped that the results from BONES will be able to guide further research into ON in young people with ALL and LBL. A greater understanding of the timing of lesion development is crucial when considering interventions to prevent or treat osteonecrotic lesions, and a recognition of those patients for whom intervention is of greatest benefit would allow targeted individualised therapy. At present there is limited data available about the subjective experience of patients who develop ON, and it is hoped that the results from the c-HAQ, together with the objective physiotherapy assessment, will give

us greater insight into the patient journey. Validation of an assessment tool developed for this specific patient population would be of value in a larger study. Further analysis on the full dataset will include the use of Chi-squared tests and multivariable logistic regression models, to determine differences between groups adjusting for a relevant set of confounders identified using causal inference methods [283]. Potential confounders that will be assessed include age, sex, ethnic group, IMD, treatment arm, highest white cell count, immune-phenotype, cytogenetics, phase of puberty, body mass index z-score, lipids, albumin, presence of VFs, BMD, ALP, PTH and vitamin D status. If numbers are sufficiently robust a more sophisticated ordered logistic regression analysis could be carried out using an ordered categorical outcome variable for severity of ON.

However, the question of how to prevent the development of osteonecrotic lesions, or how to treat existing lesions remains. In order to recruit sufficient numbers of patients it is likely that international collaboration is required. This would allow the development of a large cohort of patients for whom long term follow up data could be obtained, ideally with randomisation to treatment arms to ascertain efficacy of medical and/or surgical management in patients with ALL or LBL.

A number of possible interventions have been considered. Bisphosphonates have been considered as a possible therapeutic intervention, but our review of their use in patients with ALL and ON highlighted the lack of high quality evidence available [326]. Although it was suggested that intravenous bisphosphonates may be of value for management of pain, there was no evidence to suggest they impact on radiological progression. However, prophylactic use of bisphosphonates, or use in asymptomatic early grade lesions has not been studied in detail. Given that the majority of symptomatic lesions are diagnosed at grade 4 or 5, it is likely that any medical intervention will be of greatest benefit prior to the development of symptoms.

It is possible that newer agents affecting bone modelling will be of benefit to patients in the prevention of progression of ON. The RANK/RANKL/osteoprotegerin system regulates bone formation by regulating the osteoblast/osteoclast balance. One study found that the expression of RANK and RANKL genes were significantly elevated in osteonecrotic areas from femoral head biopsies of adult patients [343], and it is possible that RANKL inhibitors, such as denosumab, may be of benefit in

preventing the progression of ON, although safety data in paediatric patients is limited [344].

Hyperlipidaemia as a risk factor for the development of ON in young people with ALL has been previously discussed [143]. Although there have been conflicting reports [345], interventions to reduce elevated lipid levels could theoretically be used prophylactically to prevent ON development. One study assessed the development of ON in adult patients already using statins, who were subsequently given glucocorticoids [346]. The results suggest that statin use may prevent later development of ON [346], but there is a lack of safety data for statin use in young people receiving treatment for ALL. If statins are to be used in patients to prevent or reduce development of ON it is likely they will need to be used prophylactically, potentially prior to the development of any osteonecrotic lesions.

Optimal surgical management remains unclear. Very long term follow up data would be of particular benefit in understanding the outcomes following different types of arthroplasty, including the need for subsequent revision surgery, and pilot work to determine this is now underway. The role of core decompression has been discussed. It is clear that a prospective study with larger patient numbers would be of value. It is possible that patients may receive benefit from core decompression if it was performed early in the evolution of lesions, but given the high rates of spontaneous resolution of asymptomatic lesions [132], surgical intervention in asymptomatic ON is controversial.

There is on-going active research assessing the value of implantation of autologous bone-marrow cells at the time of core decompression in patients with ON [347, 348], and the use of mesenchymal stem cells (MSCs) for tissue repair in ON is biologically plausible. There is evidence that there is decreased number and activity of MSCs in osteonecrotic areas [349], and it is recognised that MSCs have the potential to provide osteogenic precursors to areas of necrosis [349]. There have been a number of randomised studies comparing implantation of autologous bone-marrow mononuclear cells at the time of core decompression with core decompression alone. These have been conducted in adult patients with ARCO grade 1 or 2 ON [347, 348], with results suggesting a significant reduction in pain and joint symptoms, and significantly improved joint survival. However, these studies were in a different patient population, all of whom had early grade ON, limiting its applicability to our patient group. There has been one study which described

the use of locally implanted autologous mesenchymal stem cells combined with core decompression in 2 adolescents with ALL and bilateral femoral head ON, ARCO grade 4 [350]. In this report, at 4 year follow up, the patients were no longer symptomatic and showed improvement in range of movement, pain and functional impairment. This report is limited by sample size, but the use of MSCs with core decompression may be of potential value in patients with established ON, and further research in this field is warranted.

Other therapeutic interventions for ON that have been discussed in the literature include hyperbaric oxygen therapy [351], extracorporeal shock wave therapy [352, 353] and free vascularized fibular grafting [354]. The evidence for all of these interventions is limited, with no approach demonstrating clear impact on the progression of ON in young patients with ALL.

6.4 Overall conclusion

This research demonstrates the high prevalence of ON in young people receiving treatment for ALL, with patients aged 10-20 years at diagnosis of ALL at greatest risk.

Surgical intervention for ON is common in these patients, with hip replacements one of the most frequent surgical procedures required. This has long term implications for both patients and the healthcare service as a whole.

Patients typically have advanced ON at the point of symptom development, and it is likely that interventions to prevent the development of severe ON will need to be initiated when lesions are asymptomatic.

A cohort study has been developed that will allow us to gain further understanding about the development of symptomatic and asymptomatic osteonecrotic lesions and vertebral fracture risk in this population. As results from this study emerge, it is hoped that the outcomes will shape future research and clinical interventions in this area.

Appendices

Appendix 1. Chemotherapeutic agents used during treatment in UKALL2011 in those eligible for BONES:

Induction:

dexamethasone 6mg/m²/day orally for 28 days (maximum single dose 10mg/day)

vincristine 1.5mg/m² intravenously weekly for 2 weeks, starting on day 2 (maximum single dose 2mg)

daunorubicin 25mg/m² intravenously on days 2, 9, 16, 23

peg-aspargase 1000iu/m² intramuscular injection day 4 and 18

methotrexate 12mg intrathecal on days 1, 8, 29

6-mercaptopurine 60mg/m²/day orally from day 29 to day 35 of consolidation.

Standard BFM consolidation:

cyclophosphamide 1000mg/m² intravenously days 1 and 15

cytarabine 75mg/m²/day intravenously or subcutaneously 4 consecutive days in weeks 6,7,8,9

mercaptopurine 60mg/m²/day orally until day 28 of consolidation

methotrexate 12mg intrathecal days 1, 8, 15

Augmented BFM consolidation:

cyclophosphamide 1000mg/m² intravenously days 1, 29

cytarabine 75mg/m² IV or subcutaneously 4 consecutive days in weeks 6,7,10 and 11

mercaptopurine 60mg/m²/day for 21 days starting week 5 of induction, and again for 14 days on days 29-42

vincristine 1.5mg/m² IV days 16, 23, 44, 51 (maximum single dose 2mg)

peg-aspargase 1000 units/m² intramuscular days 16, 44

methotrexate 12mg intrathecal days 1, 8, 22

Standard interim maintenance:

dexamethasone 6mg/m²/day orally days 1-5 and days 29-33

vincristine 1.5mg/m² IV day 1, 29 (maximum single dose 2mg)

mercaptopurine 75mg/m²/day orally days 1056

methotrexate 20mg/m² orally once/week on week 11, 12, 14, 15, 16, 18, 19

methotrexate 12mg intrathecal days 15, 43

Protocol M:

mercaptopurine 25mg/m²/day orally days 1-56

methotrexate 5g/m² intravenously days 8, 22, 36, 50

folinic acid 15mg/m² intravenously 42,48 and 54 hours after start of methotrexate infusion

methotrexate 12mg intrathecal days 8, 22, 36, 50

Capizzi interim maintenance:

vincristine 1.5mg/m² IV days 2, 12, 22, 32, 42 (maximum single dose 2mg)

methotrexate 100mg/m² IV day 2. Escalating subsequent doses as tolerated on days 12, 22, 32, 42

peg-asparagase 1000 units/m² IM days 3, 23

methotrexate 12mg intrathecal day 1, 31

Protocol M-A:

mercaptopurine 25mg/m²/day orally days 1-49

methotrexate 5g/m² IV days 1, 15, 29, 43

folinic acid 15mg/m² IV 42,48 and 54 hours after start of methotrexate infusion

methotrexate 12mg intrathecal days 1, 15, 29, 43

pegaspargase 1000 units/m² IM days 2, 23

Delayed intensification:

dexamethasone 10mg/m²/day orally for 7 days week 20 and 22
 vincristine 1.5mg/m² intravenously days 2,9,16 (maximum single dose 2mg)
 doxorubicin 25mg/m² intravenously days 2,9,16
 pegaspargase 1000iu/m² IM day 4
 methotrexate 12mg intrathecal day 1
 cyclophosphamide 1000mg/m² intravenously day 29
 mercaptopurine 60mg/m²/day orally day 29-42
 cytarabine 75mg/m²/day intravenously or subcutaneously 4 consecutive days weeks 24,25

If delayed intensification is part of regimen C the dexamethasone is given days 2-5 and 16-22, cytarabine is given in weeks 28 and 29, and vincristine given on days 2, 9, 16, 43 and 50. Intrathecal methotrexate is also given on days 29 and 36, and pegaspargase is also given on day 43.

Maintenance:

mercaptopurine 75mg/m²/day orally throughout maintenance
 methotrexate 20mg/m² orally days 1, 8, 22, 29, 36, 43, 50, 57, 64, 71, 78

If a patient has been randomised to pulses during maintenance they also receive:

dexamethasone 6mg/m²/day orally days 1-5, 29-33, 57-61
 vincristine 1.5mg/m² IV days 1, 29 and 57 (maximum single dose 2mg)

If patient was randomised to standard or Capizzi interim maintenance they will also receive 12mg of intrathecal methotrexate on day 15 of each cycle, as will T-ALL patients presenting with a white cell count of >100x10⁹/L.

All patients are also to receive co-trimoxazole prophylaxis for PCP throughout treatment with dose depending on body surface area.

Appendix 2. Data extraction form for systematic review assessing efficacy and safety of vitamin D in children and young people with acute lymphoblastic leukaemia

Study ID

Report ID

Review author ID

Citation and contact details

Confirm eligibility for review

Reason for exclusion

Notes:

Participants

n	setting	diagnostic criteria	age	sex	country	ethnicity	length of follow up

Interventions

Specific intervention:

Intervention details (dose, frequency and duration of treatment):

Intervention integrity

Adherence	Exposure (number, length, frequency)	Participant responsiveness

ALL treatment:

Protocol used	Type of steroid	Cumulative steroid dose

Outcomes

Reported/ collected	Outcome definition	Unit of measurement

Results

Outcome of interest	Number of participants (intervention)	Number of participants (control)	Mean (intervention)	Mean (control)	Standard deviation (intervention)	Standard deviation (control)

Risk of Bias Assessment

If randomised study, please complete the Cochrane risk of bias table.

If non-randomised please complete ROBINS-I table.

If study describing adverse event, please use Loke method.

For examples of how to complete tables please refer to supplementary information.

Cochrane Risk of Bias

Entry	Judgement	Support for judgement
Random sequence generation (selection bias)		
Allocation concealment		
Blinding of participants and personnel		
Blinding of outcome (detection bias) (patient-reported outcomes)		
Blinding of outcome assessment (detection bias) (Mortality)		
Incomplete outcome data addressed (attrition bias) (Short-term outcomes (2-6 weeks))		
Selective reporting (reporting bias)		

ROBINS-I:

Confounding factors: socioeconomic status, smoking, calcium intake, BMI

Co-interventions: Type of ALL treatment inc TBI, steroid dosing, type of steroid used

Bias due to confounding	
<ul style="list-style-type: none"> ○ Is there potential for confounding of the effect of intervention in this study? 	<p>N/PN/Y/PY</p> <p>If N/PN, no further signalling questions need to be considered.</p> <p>If Y/ PY assess time-varying confounding</p>
1.2 Was analysis based on splitting participants follow up time according to the intervention received?	<p>N/PN/Y/PY/NI</p> <p>If N/PN answer questions 1.4-1.6</p> <p>If Y/PY go to question 1.3</p>
1.3. Were intervention discontinuations or switches likely to be related to factors that are prognostic for the outcome?	<p>N/PN/Y/PY/NI</p> <p>If N/PN answer questions 1.4-1.6</p> <p>If Y/PY answer 1.7-1.8</p>
1.4. Did the authors use an appropriate analysis method that controlled for all the important confounding domains?	N/PN/Y/PY/NI
1.5. If Y/PY to 1.4: Were confounding domains that were controlled for measured validly and reliably by the variables available in this study?	NA / Y / PY / PN / N / NI
1.6. Did the authors control for any post-intervention variables that could have been affected by the intervention?	NA / Y / PY / PN / N / NI
1.7. Did the authors use an appropriate analysis method that controlled for all the important confounding domains and for time-varying confounding?	NA / Y / PY / PN / N / NI
1.8. If Y/PY to 1.7: Were confounding domains that were controlled for measured validly and reliably by the variables available in this study?	NA / Y / PY / PN / N / NI
Optional: What is the predicted direction of bias due to confounding?	<p>Favours experimental /</p> <p>Favours comparator</p> <p>/ Unpredictable</p>
Risk of bias judgement	<p>Low / Moderate /</p> <p>Serious / Critical / NI</p>

2.1. Was selection of participants into the study (or into the analysis) based on participant characteristics observed after the start of intervention? If N/PN to 2.1: go to 2.4	Y / PY / PN / N / NI
2.2. If Y/PY to 2.1: Were the post-intervention variables that influenced selection likely to be associated with intervention?	NA / Y / PY / PN / N / NI
2.3 If Y/PY to 2.2: Were the post-intervention variables that influenced selection likely to be influenced by the outcome or a cause of the outcome?	NA / Y / PY / PN / N / NI
2.4. Do start of follow-up and start of intervention coincide for most participants?	Y / PY / PN / N / NI
2.5. If Y/PY to 2.2 and 2.3, or N/PN to 2.4: Were adjustment techniques used that are likely to correct for the presence of selection biases?	NA / Y / PY / PN / N / NI
Risk of bias judgement	Low / Moderate / Serious / Critical / NI
Optional: What is the predicted direction of bias due to selection of participants into the study?	Favours experimental / Favours comparator / Towards null / Away from null / Unpredictable
3.1 Were intervention groups clearly defined?	Y / PY / PN / N / NI
3.2 Was the information used to define intervention groups recorded at the start of the intervention?	Y / PY / PN / N / NI
3.3 Could classification of intervention status have been affected by knowledge of the outcome or risk of the outcome?	Y / PY / PN / N / NI
Risk of bias judgement	Low / Moderate / Serious / Critical / NI
Optional: What is the predicted direction of bias due to measurement of outcomes or interventions?	Favours experimental / Favours comparator / Towards null / Away from null / Unpredictable
4.1. Were there deviations from the intended intervention beyond what would be expected in usual practice?	Y / PY / PN / N / NI
4.2. If Y/PY to 4.1: Were these deviations from intended intervention unbalanced between groups and likely to have	NA / Y / PY / PN / N / NI

affected the outcome?	
4.3. Were important co-interventions balanced across intervention groups?	Y / PY / PN / N / NI
4.4. Was the intervention implemented successfully for most participants?	Y / PY / PN / N / NI
4.5. Did study participants adhere to the assigned intervention regimen?	Y / PY / PN / N / NI
4.6. If N/PN to 4.3, 4.4 or 4.5: Was an appropriate analysis used to estimate the effect of starting and adhering to the intervention?	NA / Y / PY / PN / N / NI
Risk of bias judgement	
Optional: What is the predicted direction of bias due to deviations from the intended interventions?	
5.1 Were outcome data available for all, or nearly all, participants?	Y / PY / PN / N / NI
5.2 Were participants excluded due to missing data on intervention status?	Y / PY / PN / N / NI
5.3 Were participants excluded due to missing data on other variables needed for the analysis?	Y / PY / PN / N / NI
5.4 If PN/N to 5.1, or Y/PY to 5.2 or 5.3: Are the proportion of participants and reasons for missing data similar across interventions?	NA / Y / PY / PN / N / NI
5.5 If PN/N to 5.1, or Y/PY to 5.2 or 5.3: Is there evidence that results were robust to the presence of missing data?	NA / Y / PY / PN / N / NI
Risk of bias judgement	Low / Moderate / Serious / Critical / NI
Optional: What is the predicted direction of bias due to missing data?	Favours experimental / Favours comparator / Towards null / Away from null / Unpredictable
6.1 Could the outcome measure have been influenced by knowledge of the intervention received?	Y / PY / PN / N / NI
6.2 Were outcome assessors aware of the intervention received by study participants?	Y / PY / PN / N / NI
6.3 Were the methods of outcome assessment comparable across intervention groups?	Y / PY / PN / N / NI

6.4 Were any systematic errors in measurement of the outcome related to intervention received?	Y / PY / PN / N / NI
Risk of bias judgement	Low / Moderate / Serious / Critical / NI
Optional: What is the predicted direction of bias due to measurement of outcomes?	Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable
Is the reported effect estimate likely to be selected, on the basis of the results, from... 7.1. ... multiple outcome measurements within the outcome domain?	Y / PY / PN / N / NI
7.2 ... multiple analyses of the intervention-outcome relationship?	Y / PY / PN / N / NI
7.3 ... different subgroups?	Y / PY / PN / N / NI
Risk of bias judgement	Low / Moderate / Serious / Critical / NI
Optional: What is the predicted direction of bias due to selection of the reported result?	Favours experimental / Favours comparator / Towards null /Away from null / Unpredictable
Overall bias	Low / Moderate / Serious / Critical / NI

Loke method for quality of studies assessing adverse events:

		Support for judgement
How were adverse events data collected?		
Were any patients excluded from the adverse effects analysis?		
Did the report give numerical data by intervention group?		
Which categories of adverse effects do the investigators report?		
Did the investigators report on all important or serious effects?		
How were these defined?		
Were the methods for monitoring adverse effects reported?		

Miscellaneous

Funding source

Key conclusions of study authors

Miscellaneous comments (study author)

References to other relevant studies

Miscellaneous comments (review author)

Appendix 3. Questionnaire distributed for UKALL2003 ON data collection

Audit of osteonecrosis in children and young adults with ALL: UKALL 2003 trial period

We would be very grateful if the following information could be provided for patients with osteonecrosis on the attached list treated during the time period Oct 2003-June 2011.

Patient Demographics

Trial Reference number.....

Date of birth (dd/mm/yyyy).....

Date of end of ALL treatment.....

Date of last follow-up.....

If available:

Patient height at diagnosis of ALL.....

Patient weight at diagnosis of ALL.....

Osteonecrosis and fracture history

Joints affected by osteonecrosis (please tick all affected joints:

	Right	Left
Shoulder		
Hip		
Knee		
Ankle		
Other (please state all other affected joints)		

Date of onset of symptoms of osteonecrosis (mm/yy).....

Symptoms.....

History of fractures? Y/N

Please detail date and site of fractures if applicable.....

Date of diagnosis of osteonecrosis.....

How was diagnosis of osteonecrosis made?

Symptoms Plain X-Ray MRI Other (please state)

Imaging

Date of initial imaging.....

Types of imaging around diagnosis: MRI/ X-ray/DXA/CT

Please attach reports separately if additional space required, indicating date and type of report. If multiple areas of osteonecrosis please attach all available diagnostic reports with dates:

Initial X-Ray report (if applicable):

Initial MRI/DEXA reports (if applicable):

Follow-up MRI/ DEXA reports if available:

Management and outcome of osteonecrosis

Management (please tick all applicable):

	Yes	No	Don't know
Steroids stopped			
Pamidronate given			
Vitamin D given			
Surgical intervention required			
Other (please state)			

If applicable, date of surgery (dd/mm/yyyy).....

Type of surgery.....

Outcome (please tick):

No long term effects	
Minimal disability- able to carry out ADL	
Significant disability- unable to carry out ADL	
Requires wheelchair	
Death	
Information not available	

If the patient has died, please indicate cause of death.....

Are you aware of any children not on our list who developed osteonecrosis during this time period? Y/N

If 'yes' please complete form A and B for these patients.

Many thanks for your help with this audit.

Appendix 4. Additional form for identification of unreported cases of ON in UKALL2003.

Additional form for patients with osteonecrosis not identified on distributed list:

Trial reference number (if applicable):

Sex:

Date of birth:

Centre (registration):

Centre (follow up):

Date of diagnosis of ALL:

Ethnicity:

Treatment arm/regimen: A B A/C B/C

Other.....

Number of delayed intensification phases: 1 2

Treatment stage at diagnosis of bone toxicity:

Induction	
Consolidation	
IM 1	
DI 1	
IM 2	
DI 2	
Maintenance	

Cycle..... Week.....

Appendix 5. UKALL2003 Trial Toxicity reporting form (page 1)

MEDICAL RESEARCH COUNCIL ALL2003 TRIAL

TOXICITY REPORTING FORM version 2 - Please see both sides

Please report (only) Grade 3 and 4 toxicity by circling or highlighting the appropriate site(s) and grade(s) of toxicity for each course.

Return to: FREEPOST RLUJ-UUUU-UUAC, Patient's name.....
 CTSU, Richard Doll Building, Consultant.....
 Old Road, Headington, Oxford OX3 7LF
 Patient reference no Centre.....

Course: Induction Consolidation IMI DI 1
 IMII DI 2 Maintenance
 Cycle Week

TOXICITY CRITERIA AND GRADING (ADAPTED FROM NCI CTC)

SITE	MEASURE	GRADE				
		0/WNL	1 (Mild)	2 (Moderate)	3 (Severe)	4 (Unacceptable)
LIVER	1. ALT	WNL	=2.5 xN	2.6-5.0 xN	5.1-20.0 xN	>20.0 xN
	2. AST	WNL	=2.5 xN	2.6-5.0 xN	5.1-20.0 xN	>20.0 xN
	3. ALK PHOS-PHATASE	WNL	=2.5 xN	2.6-5.0 xN	5.1-20.0 xN	>20.0 xN
	4. TOTAL BILI	WNL	<1.5 xN	1.5-3.0 xN	>3.0 xN	>10.0 xN
	5. LIVER-CLIN	WNL	---	---	precoma	hepatic coma
PANCREAS	1. Anylase	WNL	<1.5 xN	1.5-2.0 xN	2.1-5.0 xN	>5.0 xN
	2. Glucose mmol/l	WNL	6-10	11-15	16-25	>25/ ketoacid
RENAL	1. Urea	WNL	<1.5 xN	1.5-3.0 xN	3.1-6.0 xN	>6.0 xN
	2. Creatinine	WNL	<1.5 xN	1.5-3.0 xN	3.1-6.0 xN	>6.0 xN
	3. Blood Pressure - systolic	baseline	±10%	±20%	±30%	±40%
	4. Blood Pressure - diastolic	baseline	±5%	±10%	±15%	±20%
	5. Hematuria	neg	micro only	gross, no clots	gross + clots	trans req'd
BONE		-	-	-	symptomatic severe osteopenia	Fracture/ avascular necrosis

Appendix 6. Stata code for UKALL2003 data analysis

Development of categories:

```
gen agecat=0
```

```
recode agecat 0=1 if age>10 & age<=15
```

```
recode agecat 0=2 if age>15 & age<=25
```

```
gen ethnic=0
```

```
gen tmt=0
```

```
gen racecat=.
```

```
replace racecat=0 if race==2
```

```
replace racecat=1 if race==1
```

```
replace racecat=2 if race==3
```

```
replace racecat=3 if race>3
```

```
egen agecat4 = cut(age), at (10,16,21,25)
```

```
table agecat4, contents(min age max age)
```

```
tabulate agecat4, nolabel
```

```
tab agecat4 on
```

```
egen agecat5 = cut(age), at
```

```
(1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,  
25)
```

```
. tabulate agecat5 on, row
```

```
egen percent = mean(100*on), by(agecat4)
```

```
. egen total = sum(1), by(agecat4)
```

Logistic regression:

```
logistic on i.agecat gender
```

```
logistic on age gender
```

```
logistic on age gender c.age#i.gender
```

To run each different variable as independent variable*

logistic on i.agecat gender racecat DI

logistic on i.racecat gender agecat DI

logistic on i.gender racecat agecat DI

logistic on ib(2).DI gender agecat regcat

Univariable logistic on i.agecat

logistic on i.DI

logistic on i.gender

logistic on i.racecat

logistic on i.agecat gender i.racecat ib(2).DI

calculation of chi2

tabi 22 2265 \ 104 506 \ 44 185, chi2 expected

Calculation of contingency table for age by gender interaction:

. . table agecat4 on gender, row

Calculation of likelihood ratio for age by gender interaction:

. estimates store a

. regress on i.agecat4 gender

. estimates store b

. lrtest a b

generation of kaplan-meier curve and Cox model using Breslow's method for ties

. stset Time, failure(Event)

. sts graph, by(Intervention)

. stcox Intervention, vce(cluster TrialNumber)

Appendix 7. Directed acyclic graph codes

Graph 1: Development of osteonecrosis in patients with ALL

ALL%20treatment E @0.705,0.779

Age 1 @0.146,-0.053

BMI 1 @0.750,0.120

Ethnicity 1 @0.178,0.217

Lipid_levels U @0.628,0.403

Lipid_metabolism U @0.652,0.661

Osteonecrosis O @0.873,0.360

Physical_activity U @0.764,-0.132

Pubertal_status U @0.433,-0.033

Sex 1 @0.182,0.693

Socioeconomic_status U @0.179,0.550

Steroid_metabolism U @0.819,0.727

bone_biochemistry U @0.482,0.693

bone_mineral_density U @0.535,0.844

cytogenetics_of_ALL_treatment U @0.303,0.844

diet U @0.455,0.382

number%20of%20delayed%20intensification%20blocks 1 @0.432,0.583

ALL%20treatment Osteonecrosis

number%20of%20delayed%20intensification%20blocks

Age ALL%20treatment BMI Lipid_levels Osteonecrosis Physical_activity

Pubertal_status bone_mineral_density

number%20of%20delayed%20intensification%20blocks

BMI Lipid_levels Osteonecrosis bone_mineral_density

Ethnicity BMI Lipid_levels Lipid_metabolism Osteonecrosis Physical_activity

Pubertal_status Socioeconomic_status Steroid_metabolism

bone_mineral_density cytogenetics_of_ALL_treatment diet

Lipid_levels Osteonecrosis

Lipid_metabolism Lipid_levels Osteonecrosis

Physical_activity BMI Lipid_levels Osteonecrosis bone_mineral_density

Pubertal_status BMI Lipid_levels Lipid_metabolism Osteonecrosis

Physical_activity bone_mineral_density

Sex ALL%20treatment BMI Lipid_levels Physical_activity Pubertal_status

bone_mineral_density cytogenetics_of_ALL_treatment

number%20of%20delayed%20intensification%20blocks

Socioeconomic_status BMI Physical_activity diet
 Steroid_metabolism Lipid_levels Osteonecrosis
 bone_biochemistry Osteonecrosis bone_mineral_density
 bone_mineral_density Osteonecrosis
 cytogenetics_of_ALL_treatment ALL%20treatment
 number%20of%20delayed%20intensification%20blocks
 diet BMI Lipid_levels bone_biochemistry
 number%20of%20delayed%20intensification%20blocks Lipid_levels
 Osteonecrosis

Graph 2: Development of grade 5 osteonecrosis in patients with
 osteonecrosis

BMI U @0.162,0.164

Initial_grade_of_ON 1 @0.104,0.120

Steroid_exposure U @0.096,0.319

activity_level U @0.349,0.190

additional_chemotherapeutic_agents U @0.305,0.318

age 1 @0.032,0.180

grade%205%20ON%2F%20THR O @0.444,0.248

pubertal_status U @0.313,0.239

sex 1 @0.017,0.282

BMI activity_level pubertal_status

Initial_grade_of_ON grade%205%20ON%2F%20THR

Steroid_exposure BMI activity_level grade%205%20ON%2F%20THR

activity_level Initial_grade_of_ON grade%205%20ON%2F%20THR

additional_chemotherapeutic_agents grade%205%20ON%2F%20THR

age BMI Initial_grade_of_ON Steroid_exposure activity_level

additional_chemotherapeutic_agents grade%205%20ON%2F%20THR

pubertal_status

pubertal_status grade%205%20ON%2F%20THR

sex BMI activity_level grade%205%20ON%2F%20THR pubertal_status

Appendix 8. Questionnaire responses according to centre

Centre	Number of responses	Number with no response
Addenbrookes	9	4
Alder Hey Children's hospital	6	0
Beatson West of Scotland	1	5
Birmingham Children's Hospital	27	0
Bristol Royal Hospital for Children ¹⁰	10	0
Christie Hospital NHS Trust	9	0
Clatterbridge Centre for Oncology	1	0
Great Ormond Street Hospital	14	0
Guy's and St Thomas's	0	2
James Cook University Hospital	0	1
Leeds General Infirmary	24	0
Leicester Royal Infirmary	8	0
Milton Keynes General NHS Trust	0	1
Northampton General Hospital	2	1
Nottingham City Hospital	0	2
Nottingham University Hospital	6	0
Our Lady's Hospital for Sick Children	16	0
Oxford Radcliffe Hospitals	13	0
Poole Hospital	0	1
Queen Elizabeth Hospital	0	1
Royal Aberdeen Children's Hospital	2	0
Royal Belfast Hospital	1	0
Royal Devon and Exeter Hospital	0	1
Royal Hallamshire Hospital	1	0
Royal Hospital for Sick Children	9	0
Royal Manchester Children's Hospital	23	2
Royal Marsden Hospital	16	1
Royal Shrewsbury Hospital	1	0
Royal Victoria Infirmary	15	0
Royal Wolverhampton Hospital	0	1
Sheffield Children's Hospital	5	0
Southampton University Hospital Trust	17	0
Taunton and Somerset NHS Trust	2	0
University College Hospital	3	1
University Hospital Coventry	0	1
University Hospital of North Staffs	4	0
University Hospital of Wales	9	2
Western General Hospital	1	0
Wexham Park Hospital	0	1
Yorkhill NHS trust	9	0
Total	264	28

Appendix 9. Questionnaire for surgical sub-study of UKALL2003 patients

Audit of surgical interventions for osteonecrosis in children and young adults with ALL: UKALL2003

Trial reference number:

NHS number:

Date of birth (dd/mm/yyyy):

Number of MR images: _____

Number of CDs enclosed: _____

Operation notes enclosed:

Orthopaedic letters enclosed:

Current mobility status of patient if known:

Requiring wheelchair/ requiring crutches/ requiring frame/ returned to full mobility

Date of information _____

Current pain status of patient if known:

No pain/ occasional pain relief required / regular pain relief required

Date of information _____

Appendix 10. Study protocol for British OsteoNEcrosis Study. Version 5.

BONES: The British OsteoNEcrosis Study: A prospective multi-centre study to examine the natural history of osteonecrosis in older children, teenagers and young adults with acute lymphoblastic leukaemia

Aims

The aim of this research is to examine the natural history of osteonecrosis in older children, teenagers and young adults with acute lymphoblastic leukaemia within the UK.

Objectives

The objective is to establish a prospective, multi-centre study for older children, teenagers and young adults which can address the following questions:

- What is the incidence of osteonecrosis in older children, teenagers and young adults being treated for acute lymphoblastic leukaemia (ALL) in the UK at different time points in their treatment?
- What are the risk factors for progression and the development of symptomatic osteonecrosis in this population?
- Are there specific radiological features that predict for either progression or regression in those with asymptomatic osteonecrosis?

Background

Survival from acute lymphoblastic leukaemia (ALL) has steadily increased over the last 40 years so that we now expect to cure >90% children and young people presenting with ALL. This progress shifts the entire treatment paradigm so that the goal moves beyond simply cure to returning the young person to a normal life. The biggest barrier to this is the burden of treatment associated toxicity and attention internationally is now turning to this.

Osteonecrosis (previously also referred to as avascular necrosis, ischaemic necrosis and aseptic necrosis) is one of the most devastating complications seen in older children and teenagers treated for ALL, and can cause significant long term morbidity.

However, despite increasing concern about osteonecrosis, our understanding is limited. Historically, information about osteonecrosis has not been well captured in previous studies of ALL - either in the UK or in other countries. This partly reflects lack of good definitions and piecemeal reporting. These deficiencies have been acknowledged and there is now an international will to address them. The starting point for this is standardisation of definitions, for which we can use the The National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) version 4[355], which will allow future comparison (see appendix 1). It is imperative that we maximise the potential of the current UK study, UKALL 2011, to further understanding of osteonecrosis in this population.

Osteonecrosis is one of the most debilitating complications seen after or during treatment for ALL, and is mostly an iatrogenic complication that has been attributed mostly to increased use of glucocorticoids[356]; asparaginase, high dose methotrexate and cyclophosphamide have also been implicated. Development of osteonecrosis appears to be multifactorial, but is being seen more commonly in patients as survival improves and high dose steroids have become imbedded in treatment regimens. Osteonecrosis occurs when there is bone ischaemia and infarction caused by temporary or permanent disruption to the blood supply and in ALL typically affects the femoral head, humeral head, knee, shoulder and ankles. Glucocorticoids predispose to the development of osteonecrosis in a number of ways, with proposed aetiologies including:

- Creation of a hypercoagulable state with endothelial cell apoptosis and development of microthrombi;
- Suppression of osteoblasts and apoptosis of osteocytes impairing the bone repair process;
- Stimulation of intramedullary lipocyte proliferation and hypertrophy resulting in increased intraosseous pressure.

These factors combine to compromise blood circulation to the bone leading to cell death in a self-perpetuating cycle[357].

The most comprehensive prospective study to examine osteonecrosis in children with ALL examined 364 patients and reported a cumulative

incidence of 72%, of which 18% had symptomatic osteonecrosis [132]. Symptomatic osteonecrosis was associated with a low serum albumin and high serum cholesterol, both of which were also associated with ACP1 polymorphisms. Severe osteonecrosis was associated with poor dexamethasone clearance. There are many more reports which rely on proactive reporting to the study centre, with no identification of asymptomatic osteonecrosis, and as expected these tend to give far lower incidences. These range from 0.67% [116] to 15% [123]. The UK data suggests that 4% had symptomatic osteonecrosis in UKALL 2003 [128], but it is recognised anecdotally that many patients with symptomatic osteonecrosis were not reported by clinicians in UKALL 2003.

Despite the variation in the reported incidence across the different study protocols, there is striking agreement in some of the risk factors for the development of osteonecrosis, with significant controversy in others. Age has consistently been associated with increased risk with symptomatic necrosis, with patients aged <10 years at diagnosis at much lower risk of development of osteonecrosis [132]. The significance of female sex as a risk factor for development of osteonecrosis is less clear. A number of studies found it was a risk factor while it appeared to be non-significant in other studies even when similar treatment regimens were used [111]. Even in groups with highest rates of osteonecrosis there are disparate results - the CCG study reported the disorder more frequently in females [119], whilst no gender difference were found in the DFCI ALL consortium [127] and studies at SJCRH [140]. In the study by Mattano in 2000 [119] the gender difference was greatest in the 10-15 year age group, with 3 year rates of 19.2% for females and 9.8% for males.

Ethnicity is notoriously difficult to capture. White race was found to be a risk factor in a number of studies, but not in others [117, 119, 132].

A number of candidate genes have been proposed. In the prospective study by Kawedia et al [132] single nucleotide polymorphism (SNP) genotyping was performed. After adjustment for age and treatment arm 423 SNPs were associated with symptomatic osteonecrosis, of which 27 were associated with low albumin or high cholesterol. The top 4 SNPs were in the SH3YL1-

ACP1 gene locus. ACP1 is associated with serum cholesterol and triglyceride levels [191], and regulates osteoblast differentiation [192]. Higher serum cholesterol and lower serum albumin have been associated with grade 2-4 osteonecrosis, suggesting that ACP1 may act via multiple mechanisms to affect bone homeostasis.

Dexamethasone, which is now the steroid of choice in the UK protocols, in view of its superiority over prednisolone in reducing central nervous system relapse, may be associated with an increase in osteonecrosis compared with prednisolone.

Mattano et al [105] reported higher incidence of osteonecrosis in paediatric patients with ALL treated with dexamethasone during induction phase than in those treated with prednisone (11.6% and 8.7%, respectively). This difference between these types of corticosteroids was observed only in patients' age 13 years or older, suggesting that older children may be more vulnerable to the effect of dexamethasone. Similarly, 11% of children treated with dexamethasone developed osteonecrosis in one UK report compared with only 3.5% those on prednisolone [114]. However, a much larger prospective study analysing results from UKALL97 and UKALL97/99 [100] found no excess of ON in the dexamethasone arm of the trial, but only assessed NCI grade 3 or 4 toxicity, so the impact of dexamethasone versus prednisolone in development of osteonecrosis remains unclear.

In the current UKALL 2011 study there is an upfront randomisation to standard versus short course dexamethasone. Standard dexamethasone consists of 4 weeks of dexamethasone 6mg/m² with a further weaning week. Short course dexamethasone consists of two weeks of dexamethasone 10mg/m². This is given for the first two weeks consecutively in children <10 years old, or split so that it is given for weeks 1 and 3 in older children and those with Down syndrome. The CCG1961 trial evaluated components of therapeutic intensification in high-risk patients (white cell count $\geq 50 \times 10^9$ and/or age ≥ 10 years). It was found that use of alternate week rather than continuous dexamethasone during delayed intensification in high risk ALL patients results in a 2-fold reduction in the relative risk of symptomatic osteonecrosis among rapid responders aged

≥10years, and particularly those over the age of 16 years. There was a four-fold reduction among those randomised to intensified therapy, despite those with alternate week dexamethasone having a higher total dexamethasone exposure. The incidence of ON was lower among slow responders age ≥ 10 years assigned to double delayed intensification with alternate-week dexamethasone when compared to a similar cohort on the CCG1882 trial [119] who were assigned to two delayed intensification phases with continuous dexamethasone (11.8% versus 23.2%), and could indicate that in this particular patient population dosing manner supersedes cumulative exposure. UKALL 2011 offers the first opportunity in the UK to examine the effects on osteonecrosis toxicity of short compared with standard dexamethasone.

It is recognised that osteonecrosis may regress, although the reasons for this are not understood. It is possible that some radiological changes interpreted as representing steroid associated osteonecrosis are in fact changes which have been present at diagnosis and which are a consequence of the original leukaemia. In the prospective study of 364 children[132], 39% had osteonecrosis changes on their initial MRI, but were asymptomatic. The majority of this group, 74%, did not go on to develop symptomatic osteonecrosis. The current radiological classifications use a multi-modal approach combining scores for clinical, X-ray, MRI and in some cases bone scan findings. They were developed specifically for changes in the femoral head, over 20 years ago and in an entirely different patient population.

In addition to using internationally agreed standard definitions for osteonecrosis (appendix 1), this study will provide the data needed to develop a radiological classification which correlates with clinical status.

Given the very significant morbidity associated with osteonecrosis it is imperative that the opportunity afforded by the UKALL study to examine this is maximised. Only once this is done can meaningful intervention studies to try to reduce the burden of osteonecrosis be initiated. Osteonecrosis should not be a price that young people pay for cure.

Method

Participants

Children, teenagers or young adults between the age of 10 (including the day of the 10th birthday) and 24 years 364 days (at the time of diagnosis) with a first diagnosis of acute lymphoblastic leukaemia or lymphoblastic lymphoma (T-NHL or Smlg negative precursor B-NHL) diagnosed under standard criteria are eligible for BONES. Written informed consent is required for all patients.

Recruitment

Patients will be recruited locally by the primary treatment centre.

Target recruitment

The recruitment target is 50 patients over a 2 year period, which is based on an anticipated ascertainment target of 75%. This is an observational study and there is therefore no relevant power calculation.

Data collection

Information will be collected on basic demographics, presenting features and diagnosis at initial recruitment (see appendix 2). Further data will be collected at 4 subsequent time-points detailed below to ascertain treatment and response, along with results of relevant investigations performed (see appendix 3). The clinician completing the form will access investigation results from the patient's medical records. Clinical information collected in clinic/ hospital will include height, weight and phase of puberty. At each time point (5 in total) further data will be collected, including MR imaging of lower limbs, physiotherapy assessment using a structured assessment tool, and routine clinical and biochemical information(see appendices 4, 5 and 6). Bone mineral density and lateral vertebra assessment will be assessed at diagnosis and annually to a total of 4 assessments.

Investigations

The results of the following investigations will be collected:

At diagnosis /earliest results obtained during induction- highest white cell count, immunophenotype, cytogenetics, molecular results; albumin; lipid profile; vitamin D level, bone profile (calcium, phosphate, PTH, ALP)

At the end of induction (results nearest to day 29) - MRD result, flow cytometry from end of induction bone marrow; albumin; lipid profile

V5. 02/10/2017

DXA scans results (performed at diagnosis and annually) – lumbar spine bone mineral apparent density (measured in AP direction L1-4) Z-scores, and total body less head Z-scores. Vertebral fractures would be assessed with DXA lateral vertebral assessment of thoracic and lumbar vertebra (T4-L4 if possible), using the Genant semi-quantitative method. If DXA VFA is not available, lateral thoracolumbar spine radiographs can be used instead and assessed using the same method.

Pelvic X-rays and full joint assessment via MRI which are performed if significant problems are identified by the clinical team, according to orthopaedic opinion.

Investigations specific to patients recruited into the study:

At the following time-points, patients recruited into the study will have additional assessment:

- Within 4 weeks of diagnosis
- At the end of delayed intensification
- One year after the start of maintenance
- Two years after the start of maintenance
- Three years after the start of maintenance

The additional assessment will include:

MRI of the hips, knees and ankles. These should comprise of unenhanced coronal T1 and STIR images as a minimum protocol. Knees and ankles can be imaged together. Where further information of a specific joint is needed pre-treatment additional sequences in different planes could be performed at the discretion of the participating centre.

Physiotherapy assessment, including completion of patient questionnaire.

In centres where annual DXA and lateral vertebral assessment is not standard of care, additional annual assessments will be requested where facilities exist.

The MRI images obtained are not routine MRI scans, as they are being done according to a study protocol developed for BONES, and are not for local interpretation. Local reports should simply say that images are for trial purposes only. If a significant abnormality (not osteonecrosis) is found when images are centrally reviewed, information will be fed back to the local centre. In the event of the development of symptomatic osteonecrosis, which is diagnosed locally, the patient should be managed according to local

V5. 02/10/2017

protocols and at the discretion of their own consultant (see appendix 7). Information on treatment and outcomes will be collected.

Radiological review

A central review panel consisting of Paediatric Radiologists with an interest in paediatric haematology will review each MRI in order to agree the grade of osteonecrosis and noting specific features according to the study radiology *proforma*.

There will also be retrospective central analysis of DXA and lateral vertebral assessment results. Vertebral fracture prevalence will be assessed on lateral vertebral assessment using the Genant semi-quantitative method.

Data management

Information will be collected centrally at the University of Leeds.

Local data management

Local clinician to complete forms at each time point.

Local physiotherapist to collect questionnaire data, and complete physiotherapy assessment form.

Both forms to be anonymised locally, with only trial number, initials and date of birth (in form of month/year) available on forms.

PI at local centres to be custodians of local data, and to have research file at site of personal data.

Trial centre to send separate encrypted spreadsheet of trial number, date of birth and sex to CI.

Forms and spreadsheet to be sent by secure e-mail. Consent forms to be sent to CI.

Personal data relating to study to be destroyed by PI at end of storage period (10 years).

Radiographic data

Anonymised images of MRI scans to be put onto CD, (only trial number on disk).

Anonymised DXA scans and lateral vertebral assessment images to be put onto CD (only trial number on disk) and sent to the Chief Investigator

Central data management

MRI and DXA CDs, forms and consent forms to be secured in locked filing cabinet in University of Leeds, in secure room. Only CI and members of research team to have access to this filing cabinet.

Electronic database to be created with trial numbers, date of birth (mm/yy), sex and of investigations/questionnaires.

Database to be stored on CI University M drive, a secure, password protected, University of Leeds server. A copy will be held by one of the MD research supervisors (Dr Feltbower) on their secure password protected University of Leeds server, and only available to relevant members of the research team. They will also provide the long term storage of data, after completion of student research time.

CI to be responsible for deleting data from database at end of storage period.

Statistical analysis

Epidemiology Unit located within the University of Leeds.

Participant reimbursement of expenses

Patients or their parents will be reimbursed for excess travel expenses. This will be reimbursement of public transport expenses, or car mileage (24p/mile) to a maximum of £20/ journey. Patients can claim travel expenses through petty cash arranged locally or equivalent local arrangements.

BONES protocol Appendix 1. Definition of osteonecrosis

The National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 defines ON as 'a disorder characterised by necrotic changes in the bone tissue due to interruption of blood supply. Most often affecting the epiphysis of the long bones, necrotic changes result in the collapse and the destruction of the bone structure'.

Grade	
1	Asymptomatic; clinical or diagnostic observations only, intervention not indicated.
2	Symptomatic; limiting instrumental ADL
3	Severe symptoms; limiting self care ADL; elective operative intervention indicated
4	Life-threatening consequences; urgent intervention indicated

BONES protocol Appendix 2. Form to be completed at initial recruitment

Initials _____
 Date of birth _____
 Trial Number _____
 Sex male/female/prefer not to say
 Date of initiation of therapy _____
 Ethnicity _____
 Recruiting centre _____
 Patient postcode _____
 Highest white cell count _____ x 10⁹/l date _____
 Immunophenotype _____
 Cytogenetics _____
 Molecular results _____

Height (cm) _____ Weight (kg) _____

Pubertal Status: Pre-pubertal/in puberty/completing puberty

	Pre-puberty (Tanner stage 1)	In Puberty (Tanner stage 2-3)	Completing Puberty (Tanner stage 4-5)
Girls	If all of the following: No signs of pubertal development	If any of the following: Any breast enlargement pubic or axillary hair	If all of the following Started periods with signs of pubertal development
Boys	If all of the following: High voice and No signs of pubertal development	If any of the following: Slight deepening of the voice Early pubic or axillary hair growth Enlargement of testes or penis	If any of the following: Voice fully broken Facial hair Adult size of penis with pubic and axillary hair

V5. 02/10/2017

IRAS Project ID: **185365**

Hepatomegaly yes / no

Splenomegaly yes / no

Palpable lymphadenopathy yes / no

Duration of symptoms before diagnosis _____

Was bone pain present at diagnosis? yes / no

Please document units for all available blood test results:

Blood test	Result	Date
Serum albumin		
HDL		
LDL		
Cholesterol		
Triglyceride		
25-hydroxyvitamin D		
PTH		
Alkaline phosphatase		
Calcium		
Phosphate		

Completed by: _____ date _____

BONES protocol Appendix 3. Form to be completed at day 29 of induction

Trial number _____

Patient initials _____

Date of day 29 of induction _____

Recruiting centre _____

Treatment regimen for induction A / B

Treatment regimen for consolidation A / B / C

If changed, why was this? _____

Flow cytometry results at end of induction

MRD status at end of induction low / high / not able to be assessed

Please document units for all available blood test results with units as close to day 29 as possible:

Blood test	Result	Date
Serum albumin		
HDL		
LDL		
Cholesterol		
Triglyceride		
25-hydroxyvitamin D		
PTH		
Alkaline phosphatase		
Calcium		
Phosphate		

V5. 02/10/2017

IRAS Project ID: 185365

If vitamin D was low, has this been treated? yes / no

If yes, please document treatment_____

Date of induction MRI_____

Completed by: _____ date _____

BONES protocol Appendix 4. Form to be completed and sent with relevant images at the end of delayed intensification, 1 year after start of maintenance, 2 years after start of maintenance, 3 years after start of maintenance

Trial number _____

Patient initials _____

Recruiting centre _____

Timepoint (please circle and date)

Timepoint	Date
end of delayed intensification	
1 year after start of maintenance	
2 years after start of maintenance	
3 years after start of maintenance	

Treatment regimen for interim maintenance

A standard interim maintenance

A high dose methotrexate

B standard interim maintenance

B high dose methotrexate

C Capizzi

C high dose methotrexate

Treatment regimen for maintenance vincristine/dexamethasone pulses

no pulses

Have there been any treatment modifications yes / no

V5. 02/10/2017

IRAS Project ID: **185365**

If yes, please provide further details _____

Please document units for all available blood test results:

Blood test	Result	Date
Serum albumin		
HDL		
LDL		
Cholesterol		
Triglyceride		
25-hydroxyvitamin D		
PTH		
Alkaline phosphatase		
Calcium		
Phosphate		

At the time of each scan:

Height (cm) _____ Weight (kg) _____

Pubertal status: Pre-pubertal/in puberty/completing puberty

	Pre-puberty (Tanner stage 1)	In Puberty (Tanner stage 2-3)	Completing Puberty (Tanner stage 4-5)
Girls	If all of the following: No signs of pubertal development	If any of the following: Any breast enlargement pubic or axillary hair	If all of the following Started periods with signs of pubertal development
Boys	If all of the following: High voice and No signs of pubertal development	If any of the following: Slight deepening of the voice Early pubic or axillary hair growth Enlargement of testes or penis	If any of the following: Voice fully broken Facial hair Adult size of penis with pubic and axillary hair

V5. 02/10/2017

IRAS Project ID: **185365**

Has there been a diagnosis of osteonecrosis since the last report? yes / no

If yes, when was this? _____

Which joints are affected? _____

Which of the following have occurred:	steroids stopped	yes / no
	mobility problems	yes / no
	core decompression	yes / no
	joint replacement	yes / no

Has a DXA/ lateral vertebral assessment been performed in the last year?
yes / no

If yes, please attach report and send anonymised images.

Have bisphosphonates been used? yes / no

If yes, then please give details regarding start date, type, dose and frequency of treatment

Completed by : _____ date _____

Please also attach physiotherapy assessment and send anonymised MRI images on disk to Chief Investigator

BONES protocol Appendix 5. Subjective physiotherapy assessment

At physiotherapy assessment:

For completion by physiotherapist:

Trial number:	Patient initials:
Recruiting centre:	Date:

For completion by participant



BONES

British OsteoNEcrosis Study

Activity Levels

On a typical day, on average how many hours of the day are you active for e.g. walking, playing, exercisinghours

Mobility

Since you were last seen (if relevant), were you told to continue to fully/ partially or not weight bear? Full/Partial/None

If you use a walking aid, what hand do you use it in? Right/Left/Both

If you use a walking aid, how long have you been using it for?

If you use a wheelchair, when going out, how often do you use it? Always/ Usually/ Occassionally/ Rarely/ Never?

V5. 02/10/2017

IRAS Project ID: **185365**

<h1>C.H.A.Q.</h1> <h2>Childhood health assessment questionnaire</h2>						
Patient Name:		DOB	Date:			
<ul style="list-style-type: none"> We are interested in learning how a child or young person's long term illness affects his / her ability to function in daily life. This will help the assessment in clinic. This form can be completed by the child / young person <u>themselves</u> or their parent or <u>carer</u> For the following questions, please tick one response which best describes the young person's / child's function OVER THE LAST WEEK PLEASE ONLY NOTE THOSE DIFFICULTIES WHICH ARE DUE TO THE LONG TERM ILLNESS Please note that there are 2 pages and that for very young children the answer <u>to</u> many questions will be 'Not Applicable' 						
DRESSING & PERSONAL CARE		Without ANY Difficulty	With SOME Difficulty	With MUCH Difficulty	UNABLE To Do	Not Applicable
- Dress, including tying shoelaces and doing buttons?		<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
- Shampoo hair?		<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
- Remove socks?		<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
- Cut fingernails?		<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
GETTING UP						
- Stand up from a low chair or floor?		<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
- Get in and out of bed or stand up in a cot?		<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
EATING						
- Cut own meat?		<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
- Lift a cup or glass to mouth?		<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
- Open a new cereal box?		<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
WALKING						
- Walk outside on flat ground?		<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
- Climb up five steps?		<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Please tick any AIDS or DEVICES that are usually needed for any of the above activities:						
Walking	<input type="checkbox"/>	Devices used for dressing (button hook, zip pull, <u>long-handled</u> shoe horn, etc.)				<input type="checkbox"/>
Walking Frame	<input type="checkbox"/>	Build up pencil or special utensils				<input type="checkbox"/>
Crutches	<input type="checkbox"/>	Special or built up chair				<input type="checkbox"/>
<u>Wheechair</u>	<input type="checkbox"/>	Other (Specify:)				<input type="checkbox"/>
Please tick any categories for help is usually needed from another person BECAUSE OF PAIN OR ILLNESS:						
Dressing and personal care	<input type="checkbox"/>	Eating			<input type="checkbox"/>	
Getting up	<input type="checkbox"/>	Walking			<input type="checkbox"/>	
1990 © Original version <u>singh</u> G et al. 1998 © Cross-cultural version <u>Woo P, Murray P, Nugent J</u>				PLEASE TURN OVER		

HYGIENE	Without ANY Difficulty	With SOME Difficulty	With MUCH Difficulty	UNABLE TO DO	Not Applicable
- Wash and dry entire body?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
- Take a bath (get in and get out)?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
- Get on and off the toilet or potty?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
- Brush teeth?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
- Comb / brush hair?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
REACH					
- Reach and get down a heavy object such as a large game or books from above?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
- Bend down to pick up clothing or a piece of paper from the floor?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
- Pull on a jumper over head?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
- Turn neck to look back over shoulder?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
GRIP					
- Write or scribble with a pen or pencil?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
- Open car doors?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
- Open jars which have been previously opened?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
- Turn taps on and off?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
- Push open a door when need to turn a door knob?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
ACTIVITIES					
- Run errands and shop?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
- Get in and out of a car or toy car or school bus?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
- Ride bike or tricycle?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
- Do household chores (e.g. wash dishes, take out rubbish, hoovering, gardening, make bed, clean room)?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
- Run and play?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Please tick any AIDS or DEVICES that are usually needed for the following activities:					
Raised toilet seat	<input type="checkbox"/>	Bath rail	<input type="checkbox"/>		
Bath seat	<input type="checkbox"/>	Long-handled appliances for reach	<input type="checkbox"/>		
Jar opener (for jars previously opened)	<input type="checkbox"/>	Long-handled appliances in bathroom	<input type="checkbox"/>		
Please tick any categories for which help is usually needed from another person BECAUSE OF PAIN OR ILLNESS:					
Hygiene	<input type="checkbox"/>	Gripping and opening things	<input type="checkbox"/>		
Reach	<input type="checkbox"/>	Errands and chores	<input type="checkbox"/>		
PAIN: How much pain has been experienced IN THE PAST WEEK? Place a mark on the line below, to indicate the severity of the pain					
No Pain					Very severe pain
0					10
GENERAL EVALUATION: Considering all the ways affected by pain or illness, rate how the patient is doing by placing a single mark on the line below.					
Very well					Very poor
0					10
Any concerns or questions you would like to discuss?					
.....					
.....					

BONES protocol Appendix 6: Objective physiotherapy assessment

For completion by physiotherapist:

Trial number:	Patient initials:
Recruiting centre:	Date:

Gait Analysis

.....

.....

.....

.....

ROM and Muscle power

	Muscle power (0-5)	Full range of movement	If limited range of movement, please enter degree and plane of movement that is restricted
Right hip		Yes/No	
Left hip		Yes/No	
Right knee		Yes/No	
Left knee		Yes/No	
Right ankle		Yes/No	
Left ankle		Yes/No	
Right Shoulder		Yes/No	
Left Shoulder		Yes/No	

If joints are limited please comment on why below e.g pain/stiffness

.....

.....

.....

.....

Assessment completed by Print

SignedDate

BONES protocol Appendix 7. Management of osteonecrosis

Whilst this is an observational study, it is recognised from previous experience, that management advice may be sought when a young person develops osteonecrosis. The guidelines below represent the usual practice of the clinicians involved in designing the study and are in no way mandated.

Recommendations

- Asymptomatic ON detected coincidentally.

No evidence to suggest discontinuation of dexamethasone is routinely indicated in asymptomatic cases.

Monitor closely and early repeat MRI if symptomatic

Consider orthopaedic referral. The risk of collapse of the femoral head is affected by the location and extent of the necrotic lesion. All femoral head lesions which are either large or extend to the edge of the epiphysis should be referred to orthopaedic team for consideration of core decompression in order to prevent femoral head collapse. Using MRI images in both coronal and sagittal planes the Kerboul combined necrotic angle is a good MRI-based method to assess risk of hip collapse.

- Symptomatic ON.

Confirm and document duration of symptoms in affected joint/joints. Review all other joints.

Organise physiotherapy assessment.

Review vitamin D and bone profile results.

Consider continuation of dexamethasone and 6 monthly MRI screening to detect progression of ON.

For persistent/worsening symptoms or MRI progression, reduction/discontinuation of dexamethasone will need to be considered. If in doubt contact trial coordinators in these cases.

Consider orthopaedic referral (see 1c above)

Routine use of bisphosphonates can ONLY be recommended in patients with coexisting osteoporosis, defined by reduced bone mineral density and presence of low-impact fractures (ISCD Criteria) or as part of a clinical trial.

Appendix 11. Patient information sheets and consent forms. British OsteoNEcrosis Study

What if something goes wrong?

We are not expecting anything to go wrong, but if there are any problems please tell us. If you are still unhappy about the study your family can complain for you through the hospital.

What will happen to the results of the study?

The results of the study will be printed in medical magazines and may be used to change the future treatment of acute lymphoblastic leukaemia. Your name will not be mentioned.

Who is organising the study?

The study is organised by a group of experts who work together to improve treatment for children with cancer.

Who can I ask if I want to know more?

Please ask your doctor or the research nurses if you want to know anything else about this study and they will be happy to talk to you again.

You can also visit our website on:

<http://childhealth.leeds.ac.uk/bones.html>

Thank you for reading this information leaflet.

Local contact for further information:



BONES

British OsteoNEcrosis Study

Patient information sheet for
patients aged 10-12 years



V7. 20/11/2017. IRAS 185365

We would like to invite you to take part in our research study, which forms part of a student research project. Research is a way we try to find out the answers to questions. We want to see if we can understand more about how some of the medicines you are taking act. Please read this information carefully. It is up to you and your family to decide whether you take part, so it is important for you to understand why the research is being done and what it will involve. Please ask as many questions as you want, and take time to decide if you want to take part. You should not feel that you have to take part.

What is this research?

This research is trying to find out the best way to treat young people with acute lymphoblastic leukaemia (ALL) and lymphoblastic lymphoma. We already know a lot about how to treat these but the medicines that we use are very strong and can make you feel unwell. Sometimes the medicines can cause problems and this is what we call 'side effects'. One of the possible side effects is called osteonecrosis. This means that part of a bone becomes weak. Some patients may go on to need surgery. However, a lot of the time people completely recover. We want to find out why some people get osteonecrosis and other people don't, and what happens when someone has osteonecrosis.

Why have I been chosen?

Over the next 2 years, all children being treated here aged between 10 and 25 years who get leukaemia or lymphoblastic lymphoma will be asked if they would like to take part in this study.

Do I have to take part?

No. It is up to you and your family to decide if you want to take part. If you take part but then change your mind that is okay and we will not be upset with you.

What will happen if I take part?

All patients taking part in the study will have some extra pictures taken of their legs and hips with a special scanner. These are called magnetic resonance imaging (MRI) scans.

There will be five scans in total. The first will be in the next few weeks. You will then have scans at 6 months, then one year, two years and 3 years after you started maintenance treatment. For the scan you will be asked to lie on a table and the table will move you through the scanner. It is doesn't hurt, and takes around 30 minutes. The scan results are for our research only but if we see something unusual we will let the team who is looking after you know.

You will also have an appointment with a physiotherapist at roughly the same times as the scans. This will take around half an hour. Physiotherapists look at how patients are moving, and they will help us recognise if there are any problems developing with your arms or legs. They will also use some questionnaires to understand if there are any problems with your arms or legs.

As part of the study we will look at the results of some of the other tests that you will have anyway, such as blood tests and scans of your bones.



If you agree to take part in this study you will be asked to sign a form called an assent form. You will be given a copy of it with this information sheet to keep.

MRI Scanner

Will joining the research study help me?

The aim of the study is to gain information to help improve how we look after young people with ALL or lymphoblastic lymphoma in the future. We are not expecting you to directly benefit from taking part. All the extra tests are only for the study and will not change how you are managed unless we find something unexpected on the scans.

Will anyone else know I'm doing this?

The only people who will know that you are taking part in this study will be the team of doctors, nurses and researchers looking after you. We will let your GP know that you are taking part so they understand why you are having some extra tests.

What will happen if I don't want to carry on with the study?

You can stop taking part in this study at any time without giving any reason. No-one will mind if you don't want to carry on with the study.

Who can I ask if I want to know more?

Please ask your doctor or the research nurses if you want to know anything else about this study and they will be happy to talk to you again.

V7. 20/11/2017. IRAS 185365

Will anyone else know I'm doing this?

The only people who will know that you are taking part in this study will be the team of doctors, nurses and researchers looking after you. We will let your GP know that you are taking part so they understand why you are having some extra tests. Your identity during this study will be confidential and protected. When you first register for the study, you will be given a study number. This study number, along with your initials and date of birth will be used to identify your samples and the data we collect.

What will happen if I don't want to carry on with the study?

You are free to leave the trial at any time without giving a reason and this will not affect your future treatment. If you decide to withdraw from the trial you will be asked to allow us to continue collection of follow-up data (you will not need to attend more clinic appointments than is normal for your condition).

What if there is a problem?

If you wish to complain or are unhappy about any aspect of the way you have been approached or treated during the course of the study, in the first instance please contact your consultant or a member of the research team- you can use the contact numbers at the end of this sheet. If you are still unhappy, you or your family can complain through the hospital complaints department.

What will happen to the results of the trial?

Results may be published in medical and scientific journals, and presented at international conferences, but your name will not be used in any publications. If you would like to obtain a copy of the published results, please ask your doctor or nurse.

V7. 20/11/2017. IRAS 185365

Who has reviewed the trial?

This trial has been reviewed by the an independent Research Ethics Committee. Research Ethics Committees review all research to protect the safety, rights, well being and dignity of patients.

Thank you for reading this information leaflet.

For further information you can visit our website: <http://childhealth.leeds.ac.uk/bones.html>

Local contact for further information

If you require any further information please contact:

**BONES****British OsteoNEcrosis Study****Patient information sheet for patients aged 13-15 years**

We would like to invite you to take part in a trial called **BONES** (British Osteonecrosis Study), which is part of a student research project.

Before you decide whether you want to take part in the study we would like you to understand why the study is being done and what it would involve.

Please take the time to read the following information carefully and discuss it with friends, relatives, doctors and nurses if you wish. Ask us if there is anything that is not clear, or if you would like more information.

What is this research?

This research is trying to find out the best way to treat young people with acute lymphoblastic leukaemia (ALL) and lymphoblastic lymphoma. We already know a lot about these conditions, but the medicines that we use are very powerful. Sometimes the medicines can cause problems and this is what we call 'side effects'.

One of the side effects that can occur is called **osteonecrosis**. This means a part of the bone breaks down, and usually happens in joints such as the hip, knee and ankle. If osteonecrosis is severe patients may go on to need surgery. However, it is often not very severe and people can completely recover.

In our research we are trying to find out:

- What makes a person more likely to develop osteonecrosis
- When osteonecrosis develops
- What happens to patients once they develop osteonecrosis

This should help us to pick up patients who are at risk, and develop ways to try and stop them from developing severe osteonecrosis.

Why have I been invited?

You have been invited because you have been diagnosed with leukaemia or lymphoma and are aged between 10 years and 25 years. Over the next 2 years a number of hospitals around the country will be inviting children and young people diagnosed with leukaemia or lymphoma to take part in this trial.

Do I have to take part?

No. It is up to you to decide whether or not you want to take part. You can leave the trial at any time, and if you do leave your treatment for your leukaemia will not change.

V7. 20/11/2017. IRAS 185365

What will happen if I take part?

Being in the study involves scans, physiotherapy assessments and some questionnaires.

All patients taking part in the study will have some pictures taken of their legs and hips with a special scanner. These are called magnetic resonance imaging (MRI) scans. There will be 5 scans in total. The first scan will be in the next few weeks. The following scans will be at six months, then one, two and three years after you start maintenance treatment.

For the scan you will be asked to lie on a table and the table will move you through the scanner. It doesn't hurt, and will take around half an hour.

The scan results are for our research only but if we see something unusual we will let the people who are looking after you know.



MRI Scanner

You will also have an appointment with a physiotherapist at roughly the same times as the MRI scans, which will take around 30 minutes.

Physiotherapists look at how patients are moving, and they will help us recognise if there are any problems developing with your arms or legs. They will also ask you to fill in a questionnaire to see if there seem to be any problems developing.

The research team will also review your medical records and look at results of some of the other tests which you will be having as part of your treatment.

If you agree to take part in this study you will be asked to sign an assent form. You will be given a copy of it, and this information sheet to keep.

Are there any disadvantages or risks involved in taking part in this study?

If you decide to take part in this trial the leukaemia treatment you receive will be the same as if you chose not to take part.

MRI scans are painless and very safe. They do not involve radiation and there are no known side effects of an MRI scan.

What are the possible benefits of taking part?

The aim of the study is to gain information to help improve how we look after young people with ALL or lymphoblastic lymphoma in the future. We are not expecting you to directly benefit from taking part. All the extra tests are only for the study and will not change how you are managed unless something unexpected is seen on the scans.

What happens when the trial stops?

At the end of the trial all of the data that has been gathered will be examined, and the results used in to help identify patients at highest risk of osteonecrosis, and consider how this risk can be reduced. The information collected will be stored on a safe database for analysis and will only be accessed by authorised people.

Will my participation in this study be kept confidential?

During this study your identity will be protected as defined under the Data Protection Act 1998. When you are first registered onto this study you will be given a study number. This study number, along with your initials and date of birth will be used to identify the data we collect.

Only information needed for this study will be collected. All information will be strictly confidential. By taking part in the trial you will be agreeing to allow research staff to look at the trial records, including your medical records and scan images. Your medical records and all data obtained from this study will be made available to representatives of the study Sponsor and regulatory authorities. This is to make sure the information collected is an accurate reflection of the study.

The information collected will be stored on a secure database for analysis at the University of Leeds, and will only be accessed by authorised people, who have a duty of confidentiality to you. Your GP will also be informed so they understand why you will be having some extra tests. You will not be able to be identified in any report, presentation or publication arising from this trial.

What will happen to the results of the trial?

Results may be published in medical and scientific journals, and presented at international conferences, but your name will not be used in any publications. If you would like to obtain a copy of the published results, please ask your doctor or nurse.

Who has reviewed the trial?

This trial has been reviewed by the an independent Research Ethics Committee. Research Ethics Committees review all research to protect the safety, rights, well being and dignity of patients.

What will happen if I don't want to carry on with the study?

You are free to withdraw from this trial at any time without giving a reason and this will not affect your future treatment. If you decide to withdraw you will be asked to allow the continued collection of follow-up data (you will not need to attend more clinic appointments for this than normal for your condition).

Who is organising and funding the research?

This study is funded by Candlelighters charitable foundation and sponsored by the University of Leeds. No-one will receive payment for taking part in this study.

What if there is a problem?

Any concern or complaint about the way you have been dealt with during the trial or any possible harm you might suffer will be addressed. If you wish to complain or are unhappy about any aspect of the way you have been approached or treated during the course of the study, in the first instance please contact your consultant or a member of the research team- you can use the contact numbers at the end of this sheet. If you are still unhappy you can complain through the hospital complaints department.

Local contact for further information

If you require any further information please contact:



BONES

British Osteonecrosis Study

Patient information sheet for patients aged 16+ years

We would like to invite you to take part in a clinical trial run by the University of Leeds called BONES (British Osteonecrosis Study), which is part of a postgraduate research project. Before you decide whether you want to take part in the study we would like you to understand why the study is being done and what it would involve.

Please take the time to read the following information carefully and discuss it with friends, relatives, doctors and nurses if you wish. Ask us if there is anything that is not clear, or if you would like more information.

You can also visit our website:
<http://childhealth.leeds.ac.uk/bones.html>

V7.20/11/2017. IRAS 185365

What is the purpose of the study?

You have been diagnosed with Acute Lymphoblastic Leukaemia (ALL) or lymphoblastic lymphoma. The treatment is usually very successful and we are now trying to improve treatment further by investigating the side-effects that can occur during and after treatment, in order to reduce these. One of the side effects that can occur in parts of bone is called osteonecrosis. This happens when there is an interruption to the blood supply to the bone which causes changes in the bone itself, and happens most often in the hips, knees, and ankles. If osteonecrosis is severe patients need surgery. However, in many cases where it is less severe the patient may recover fully.

We know that osteonecrosis occurs more commonly in patients over 10 years of age but we don't know why some people develop it and others do not. With this study we hope to learn more about:

- What makes a person more likely to develop osteonecrosis
- When osteonecrosis develops
- What happens to patients when they develop osteonecrosis

Why have I been invited?

You have been invited because you have been diagnosed with ALL or lymphoblastic lymphoma and are aged between 10 years and 25 years. Over the next 2 years a number of hospitals in the UK will be inviting children and young people diagnosed with ALL or lymphoblastic lymphoma to take part in this trial.

Do I have to take part?

No, taking part is entirely voluntary. It is up to you to decide whether or not you want to take part. You can withdraw at any time, without giving a reason. This would not affect the rest of the care that you receive.

Will anyone else know I'm taking part?

The only people who will know that you are taking part in this study will be the team of doctors, nurses and researchers looking after you.

What will happen if I take part?

Being in the study involves scans, a physiotherapy assessment and a questionnaire. We will also look at your medical records to see the results of some of the tests you are having routinely.

We will look for signs of osteonecrosis by taking pictures of your legs and hips with a special scanner. These are called magnetic resonance imaging (MRI) scans. There will be five scans in total. The first scan will be in the next few weeks. The next scans will be at six months, then one year, two years and three years after you start maintenance treatment. For the scan you will be asked to lie on a table and the table will move through the scanner. It doesn't hurt, and will take around half an hour.

You will also have an appointment with a physiotherapist at roughly the same times as the scan, which will take around 30 minutes. Physiotherapists look at how patients are moving, and they will help us recognise if there are any problems developing with your arms or legs.

They will also ask you to complete a questionnaire to see if there seem to be any problems developing.

In some centres there will be extra imaging of bones by dual energy X-ray absorptiometry (DXA), which measures bone mineral density and assesses fracture risk. These are routinely performed in some centres, but there is not currently a national standard. We would like to look at the results of these scans, which will be performed at diagnosis and annually, to a total of 4 scans. DXA scans are very safe and painless. You would be required to lie on your side on an X-ray table as a scanner passes over you.

If you agree to take part in this study you will be asked to sign a consent form. You will be given a copy of it, and this information sheet to keep.



MRI Scanner

We can reimburse reasonable travel expenses (public transport or car mileage) which are due to being part of this study.

Are there any disadvantages or risks involved in taking part in this study?

If you decide to take part in this trial the leukaemia treatment you receive will be the same as if you choose not to participate.

MRI scans are painless and very safe. They do not involve radiation and there are no known side effects of an MRI scan. There are some cases where an MRI scan may not be recommended, because the strong magnets used during the scan can affect metal implants or fragments in the body. Please let your health care team know if you have any metal in your body. DXA scans use a very low dose of radiation (less than 2 days exposure to normal background radiation), which is much lower than standard X-ray examinations.

There is a possibility we might find something unexpected in your images. If this happens, we will notify you first and you will be referred to the appropriate specialist for further investigation.

Before any trial can start it has lots of safety checks before it can be approved. This study has undergone these checks and we hope that the trial will help improve the treatment for children and young adults with ALL and lymphoblastic lymphoma in the future.

What are the possible benefits of taking part?

The aim of the study is to gain information to improve how we look after young people with ALL or lymphoblastic lymphoma in the future. We are not expecting you to directly benefit from taking part. All the extra tests are only for the study and will not change how you are managed unless something unexpected is seen.

What happens when the trial stops?

At the end of the trial all of the data that has been gathered will be examined, and the results used in the future to help identify patients at highest risk of osteonecrosis, and consider how this risk can be reduced. Anonymous data will be kept for 10 years.

V7.20/11/2017. IRAS 185365

Before any trial can start it has to have lots of safety checks before it can be approved to become a clinical trial. This study has undergone these checks and we hope that the trial will help improve the treatment for children and young adults with ALL in the future.

What happens when the trial stops?

At the end of the trial all of the data that has been gathered will be examined, and the results used in the future to help determine patients at highest risk of osteonecrosis, and consider how this risk can be reduced. Information will be stored for 10 years, and follow-up of outcomes will be part of future studies.

Will my child's participation in this study be kept confidential?

During this study your child's identity will be protected as defined under the Data Protection Act 1998. When your child is first registered onto this study they will be given a study number. This study number, along with their initials and date of birth will be used to identify the data we collect.

Only information needed for this study will be collected. All information will be kept strictly confidential. By taking part in the trial you will be agreeing to allow research staff to look at the trial records, including your child's medical records and radiographic images. Your child's medical records and all data obtained from this study will be made available to representatives of the study sponsor and regulatory authorities. This is to make sure the information collected is an accurate reflection of the study.

We will also inform your child's GP to ensure that they are aware of the reason for the additional investigations being carried out. The information collected will be stored in an anonymised format on a secure database at the University of Leeds. This will only be accessed by authorised personnel. All individuals who have access to your child's information have a duty of confidentiality to him/her. Under no circumstances will your child be identified in any way in any report, presentation or publication arising from this trial.

What will happen if I don't want my child to carry on with the study?

If, after discussion with your child, you no longer wish for your child to continue in this study, you are free to withdraw from the study at any time without giving a reason and this will not affect his/her future treatment. If you withdraw your child from the trial you will be asked to allow the continued collection of follow-up data.

What if there is a problem?

Any concern or complaint about the way your child has been dealt with during the trial or any possible harm your child might suffer will be addressed. If you wish to complain or are unhappy about any aspect of the way you have been approached or treated during the course of the study, in the first instance please contact your child's consultant. If you are still unhappy you can contact the hospital complaints department.

What will happen to the results of the trial?

Results may be published in medical and scientific journals, and presented at international conferences, but no individual patients will be identified. If you would like to obtain a copy of the published results, please ask your doctor or nurse.

Who has reviewed the trial?

This trial has been reviewed by the an independent Research Ethics Committee. Research Ethics Committees review all research to protect the safety, rights, well being and dignity of patients.

Local contact for further information

If you require any further information please contact:



BONES

British Osteonecrosis Study

Parent Information Sheet

We would like to invite your child to take part in a clinical trial called BONES (British Osteonecrosis Study), which is part of a postgraduate research project.

Before you decide whether you want your child to take part in the BONES study we would like you to understand why the trial is being done and what it would involve. Please read the following information carefully and discuss it with friends, relatives, doctors and nurses if you wish. Ask us if there is anything that is not clear, or if you would like more information.

You can also visit our website:

<http://childhealth.leeds.ac.uk/bones.html>

V8. 20/11/2017. IRAS 185366

What is the purpose of the study?

Your child has been diagnosed with acute lymphoblastic leukaemia (ALL) or lymphoblastic lymphoma. The treatment for this is usually very successful. We are now trying to improve treatment further by investigating the side-effects that can occur during and after treatment, in order to reduce these. A serious side effect that can occur is called osteonecrosis. This is where an interruption in the blood supply to the bone causes changes within the bone. If osteonecrosis is severe, surgery may be needed. However, in many cases where it is less severe the patient can recover fully. We know that osteonecrosis is more common in patients over 10 years of age, but we don't know why some people develop it and others do not. With this study we hope to learn more about:

- What risk factors make someone more likely to develop osteonecrosis
- When osteonecrosis develops
- What happens to patients when they develop osteonecrosis

Why has my child been invited?

Your child has been invited because he/she has been diagnosed with ALL or lymphoblastic lymphoma and is aged between 10 years and 25 years. Over the next 2 years a number of hospitals around the country will be inviting children and young people diagnosed with ALL or lymphoblastic lymphoma to take part in this trial.

Does my child have to take part?

No, participation in this trial is entirely voluntary. It is up to you to decide whether or not you want your child to take part. You are free to withdraw your child at any time, without giving reason. This would not affect the standard of care that he/she receives.

Who is organising and funding the research?

This study is funded by Candlelighters charitable foundation and sponsored by the University of Leeds. No-one will receive payment for taking part in this study but we can reimburse reasonable travel expenses (public transport or car mileage) which are incurred due to being part of this study.

What will happen if my child takes part?

If you agree for your child to take part in this study you will be asked to sign a consent form. You will be given a copy of it, and this information sheet to keep. Being in the study involves scans, physiotherapy assessments and completion of a questionnaire.

We will look for signs of osteonecrosis by organising magnetic resonance imaging (MRI) scans:

- Within 4 weeks of diagnosis
- At the end of delayed intensification
- One year after the start of maintenance
- Two years after the start of maintenance
- Three years after the start of maintenance.

Scans will be of hips, knees and ankles, as these are the areas most commonly affected by osteonecrosis. MRI scans use radio-waves rather than x-rays and produces images that can be analysed on a computer. Your child will be asked to lie on a table and the table will move them through the scanner. It is a painless process, and usually takes around 30 minutes.

Your child will also have a physiotherapy assessment at each of the time points above. This will help us identify if there are any problems developing with your child's movements. This will take around 30 minutes, and will include completion of questionnaires about arm and leg movements and function.

In some centres there will be extra imaging of bones by dual energy X-ray absorptiometry (DXA), which measures bone mineral density and assesses fracture risk. These are routinely performed in some centres, but there is not currently a national standard. We would like to look at the results of these scans, which will be performed at diagnosis and annually, to a total of 4 scans. DXA scans are very safe and painless. They require the patient to lie on their back and side on an X-ray table as a scanner passes over them.

If your child takes part, the research team will also look at the results of some routine blood tests and scans, and collect some routine clinical information. This will be provided to the research team in an anonymised form by your clinical team.

What are the possible benefits of taking part?

The aim of the study is to gain information to help improve how we look after young people with ALL or lymphoblastic lymphoma in the future. We are not expecting your child to directly benefit from taking part. All the extra tests are only for the study and will not change how your child is managed unless something unexpected is picked up.

Are there any disadvantages or risks involved in my child's participation in this study?

If your child decides to take part in this study the treatment they receive will be the same as if they choose not to participate.

MRI scans are painless and very safe. They do not involve radiation and there are no known side effects of an MRI scan. There are some cases where an MRI scan may not be recommended, because the strong magnets used during the scan can affect metal implants or fragments in the body. Please let your child's health care team know if they have any metal in their body. Examples include if your child has a pacemaker, metal heart valves, or an artificial joint. If they have something metallic in their body they may still be able to take part in the study, but it is important for the medical team to be aware so they can decide on a case-by-case basis how to make things as safe as possible.

DXA scans use a very low dose of radiation (less than 2 days exposure to normal background radiation), which is much lower than standard X-ray examinations.

There is a possibility we might find something unexpected in your child's images. If this happens, we will notify you first and your child will be referred to the appropriate specialist for further investigation.

V8. 20/11/2017. IRAS 185366

Informed Consent Form (Patient aged 16 years and over)**British OsteoNEcrosis Study**

Site _____ Principle Investigator _____

Patient Trial Number _____ Trial Reference Number _____

Please initial each box

1. I confirm that I have read and understood the Patient Information Sheet (version 7, 20/11/2017) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason and without my medical care or legal rights being affected.
3. I give permission for a copy of this consent form to be sent to the research team based at the University of Leeds.
4. I understand that relevant sections of my medical notes and data collected during the trial may be looked at by individuals from the research team, regulatory authorities, Sponsors and/or NHS bodies, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records and to collect, store, analyse and publish information from this research. I understand that my name will be kept confidential.
5. If I withdraw from the study I agree to allow the continued collection of follow up data.
6. I agree for my GP to be informed about my involvement in this study
7. I agree to take part in the above study.
8. I consent for data from this study to be used in future research projects

Name of patient: _____ Date: _____ Signature: _____

Name of person taking consent: _____ Date: _____ Signature: _____

Assent Form (Patient less than 16 years of age)**British OsteoNEcrosis Study**

Site _____

Principle Investigator _____

Patient Trial Number: _____ Trial Reference Number: _____

Please initial each box

1. I confirm that I have read and understood the Patient Information Sheet (version 7, 20/11/2017) for the above study. I have had time to consider the information, ask questions and have had these answered satisfactorily.
2. I understand that I can choose whether or not to take part in this study. I can withdraw at any time without giving a reason. This will not affect my care.
3. I allow a copy of this assent form to be sent to the research team based at the University of Leeds.
4. I understand that relevant parts of my medical notes and information collected during the study may be looked at by people from the research team, or people supervising this study. I give permission for these people to have access to my records and to collect, store, analyse and publish information from this research. I understand that my name will be kept confidential.
5. If I withdraw from the study I agree to allow follow up data to still be collected.
6. I agree for my GP to be informed about my taking part in this study
7. I agree to take part in the above study.
8. I agree for data from this study to be used in future research projects

Name of patient: _____ Date: _____ Signature: _____

Name of parent/guardian: _____ Date: _____ Signature: _____

Name of person taking assent: _____ Date: _____ Signature: _____

Informed Consent Form (Parent)**British OsteoNEcrosis Study**

Site _____ Principle Investigator _____

Patient Trial Number _____ Trial Reference Number _____

Please initial each box

1. I confirm that I have read and understand the Parent Information Sheet (version 8, dated 20/11/2017) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily
2. I understand that my child's participation is voluntary and that we are free to withdraw at any time without giving any reason and without his/her medical care or legal rights being affected.
3. I give permission for a copy of this consent form to be sent to the research team based at the University of Leeds.
4. I understand that relevant sections of my child's medical notes and data collected during the trial may be looked at by individuals from the research team, regulatory authorities, Sponsors and/or NHS bodies, where it is relevant to my child's taking part in this research. I give permission for these individuals to have access to my child's records and to collect, store, analyse and publish information from this research. I understand that my child's name will be kept confidential.
5. If I withdraw my child from the study I agree to allow the continued collection of follow up data.
6. I agree for my child's GP to be informed about their involvement in this study.
7. I agree for my child to take part in the above study.
8. I consent for data from this study to be used in future research projects

Name of patient: _____

Name of parent/guardian: _____ Date: _____ Signature: _____

Name of person taking consent: _____ Date: _____ Signature: _____

Appendix 12. British OsteoNEcrosis Study ethical approval



Health Research Authority

Yorkshire & The Humber - Sheffield Research Ethics Committee

Jarrow Business Centre
Rolling Mill Road
Jarrow
Tyne and Wear
NE32 3DT

Telephone: 0207 104 8085

Please note: This is the favourable opinion of the REC only and does not allow you to start your study at NHS sites in England until you receive HRA Approval

12 July 2016

Dr Nadia L Amin
Clinical Research Fellow
University of Leeds
Room 9.88, Worsley Building
Clarendon Way
Leeds
LS2 9NL

Dear Dr Amin

Study title:	BONES: The British Osteonecrosis Study: A prospective multi-centre study to examine the natural history of osteonecrosis in older children, teenagers and young adults with acute lymphoblastic leukaemia or lymphoblastic lymphoma.
REC reference:	16/YH/0206
Protocol number:	n/a
IRAS project ID:	185365

Thank you for your letter of 25 June 2016, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to make a request to postpone publication, please contact the REC Manager, Miss Kathryn Murray, nrescommittee.yorkandhumber-sheffield@nhs.net.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Conditions of the favourable opinion

The REC favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements. Each NHS organisation must confirm through the signing of agreements and/or other documents that it has given permission for the research to proceed (except where explicitly specified otherwise).

Guidance on applying for NHS permission for research is available in the Integrated Research Application System, www.hra.nhs.uk or at <http://www.rdforum.nhs.uk>.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of management permissions from host organisations.

Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publicly accessible database within 6 weeks of recruitment of the first participant (for medical device studies, within the timeline determined by the current registration and publication trees).

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g. when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non-clinical trials this is not currently mandatory.

If a sponsor wishes to contest the need for registration they should contact Catherine Blewett (catherineblewett@nhs.net), the HRA does not, however, expect exceptions to be made. Guidance on where to register is provided within IRAS.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Ethical review of research sites

NHS sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

<i>Document</i>	<i>Version</i>	<i>Date</i>
Covering letter on headed paper [Cover letter]	1	24 June 2016
Evidence of Sponsor insurance or indemnity (non NHS Sponsors only) [Sponsor indemnity]	1	22 September 2015
GP/consultant information sheets or letters [GP information sheet]	1	28 September 2015
IRAS Application Form [IRAS_Form_11052016]		11 May 2016
IRAS Application Form XML file [IRAS_Form_11052016]		11 May 2016
IRAS Checklist XML [Checklist_25062016]		25 June 2016
Letter from sponsor [Sponsor e-mail]	1	03 February 2016
Letter from statistician [Letter from statistician]		29 September 2015
Other [Revised Question A6-1]	2	23 June 2016
Participant consent form [Consent form 16+]	3	15 June 2016
Participant consent form [Consent form parent]	3	15 June 2016
Participant consent form [Assent form]	2	15 June 2016
Participant information sheet (PIS) [PIS age 10-12]	4	14 June 2016
Participant information sheet (PIS) [PIS age 13-15]	4	14 June 2016
Participant information sheet (PIS) [PIS age 16+]	4	14 June 2016
Participant information sheet (PIS) [PIS Parent]	5	14 June 2016
Research protocol or project proposal [Protocol]	3	29 December 2015
Summary CV for Chief Investigator (CI) [CV N Amin]	1	13 November 2015
Summary CV for Chief Investigator (CI) [CV CI]	1	13 November 2015
Summary CV for student [Student CV]	1	13 November 2015
Summary CV for supervisor (student research) [Supervisor CV - S Kinsey]	1	13 November 2015
Summary CV for supervisor (student research) [CV R Feltbower]	1	13 November 2015
Summary CV for supervisor (student research) [B James CV]	1	16 May 2016
Validated questionnaire [Hip questionnaire]	1	

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

A Research Ethics Committee established by the Health Research Authority

Appendix 13. Amendments to protocol. Ethical approval.



Health Research Authority

Yorkshire & The Humber - Sheffield Research Ethics Committee

Room 001
Jarrow Business Centre
Rolling Mill Road
Jarrow
Tyne & Wear
NE32 3DT

Tel: 0207 104 8282

Please note: This is the favourable opinion of the REC only and does not allow the amendment to be implemented at NHS sites in England until the outcome of the HRA assessment has been confirmed.

12 April 2017

Dr Nadia L Amin
Clinical Research Fellow
University of Leeds
Room 9.88, Worsley Building
Clarendon Way
Leeds
LS2 9NL

Dear Dr Amin

Study title:	BONES: The British Osteonecrosis Study: A prospective multi-centre study to examine the natural history of osteonecrosis in older children, teenagers and young adults with acute lymphoblastic leukaemia or lymphoblastic lymphoma.
REC reference:	16/YH/0206
Protocol number:	n/a
Amendment number:	Substantial Amendment 1 - 12/3/17
Amendment date:	12 March 2017
IRAS project ID:	185365

The above amendment was by the Sub-Committee in correspondence.

This amendment is to gain approval for the make changes to the Protocol, patient information sheets and consent forms.

Ethical opinion

The members of the Committee taking part in the review gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

The Sub Committee did not raise any ethical issues.

Approved documents

The documents reviewed and approved at the meeting were:

Document	Version	Date
Covering letter on headed paper	Email from Nadia Amin	30 March 2017
Notice of Substantial Amendment (non-CTIMP)	Substantial Amendment 1 - 12/3/17	12 March 2017
Participant consent form [Assent Form]	Version 5	09 March 2017
Participant consent form [Parent]	Version 6	09 March 2017
Participant consent form [Ages 16+ years]	Version 6	09 March 2017
Participant information sheet (PIS) [Parent]	Version 7	09 March 2017
Participant information sheet (PIS) [Age 16+ years]	Version 6	09 March 2017
Participant information sheet (PIS) [Ages 13-15 years]	Version 6	09 March 2017
Participant information sheet (PIS) [Ages 10-12 years]	Version 6	09 March 2017
Research protocol or project proposal	Version 4	12 March 2017

Membership of the Committee

The members of the Committee who took part in the review are listed on the attached sheet.

Working with NHS Care Organisations

Sponsors should ensure that they notify the R&D office for the relevant NHS care organisation of this amendment in line with the terms detailed in the categorisation email issued by the lead nation for the study.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

We are pleased to welcome researchers and R & D staff at our Research Ethics Committee members' training days – see details at <http://www.hra.nhs.uk/hra-training/>

16/YH/0206: Please quote this number on all correspondence

Yours sincerely

pp



Professor Basil Sharrack
Chair
E-mail: nrescommittee.yorkandhumber-sheffield@nhs.net

Enclosures: *List of names and professions of members who took part in the review*

Copy to: *Anne Gowing, Leeds Teaching Hospitals NHS Trust*



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Please note: This is the favourable opinion of the REC only and does not allow the amendment to be implemented at NHS sites in England until the outcome of the HRA assessment has been confirmed.

14 February 2018

Dr Nadia Laila Amin
9 Ayresome Avenue
Leeds
LS8 1BB

Dear Dr Amin

Study title:	BONES: The British Osteonecrosis Study: A prospective multi-centre study to examine the natural history of osteonecrosis in older children, teenagers and young adults with acute lymphoblastic leukaemia or lymphoblastic lymphoma.
REC reference:	16/YH/0206
Protocol number:	n/a
Amendment number:	Substantial Amendment 5, 20/11/2017
Amendment date:	17 January 2018
IRAS project ID:	185365

The above amendment was reviewed by the Sub-Committee in correspondence.

Summary of Amendment

Submission of this amendment was to amend the protocol to remove collection of blood samples P1NP, CTX and bone specific alkaline phosphatase, as well as removal of collection of samples for genetic analysis with additional specification for data collection of routine markers of bone metabolism (calcium, phosphate, PTH, ALP). Genetic analysis had been removed from the study as this was to be confirmed, and will be discussed and consented as a separate study when confirmation of viability was obtained. The protocol had minor changes regarding terminology for DXA imaging of vertebra. The initial recruitment period to the study had been extended to four weeks after diagnosis of ALL or lymphoblastic lymphoma.

The protocol had additional information regarding the requesting of annual DXA imaging in centres where facilities exist. It had become apparent that there was a variation in standard of care across the UK regarding the use of DXA imaging in patients with ALL and lymphoblastic lymphoma, and this sentence was to clarify the protocol. The protocol had further clarification regarding central assessment of DXA imaging. The protocol had been amended to include information regarding reimbursement of travel expenses for patients participating in the study. Minor changes had been made to clinician and physiotherapy forms to reduce collection of unnecessary patient information. Postcode collection had been added to the data collection form to allow analysis of deprivation score of participants, using the Index of Multiple Deprivation. Changes had been made to the clinician forms to collect additional routine biochemical information that would provide an understanding of bone metabolism. Patient information sheets had been amended to remove mention of additional blood sampling for markers of bone turnover or genetic analysis. Parent and 16+ information sheets included clarifying information regarding variation in national practice of DXA imaging, and routine use for patients in BONES where facilities exist. The parent and 16+ information sheets had been amended to include information regarding reimbursement of reasonable travel expenses incurred from participation in BONES. Consent and assent forms had been amended to include the new version of patient information sheets and removal of consent for samples for genetic analysis. A thank you letter had been developed, which was to be sent to patients after they had agreed to participate in the study.

Ethical opinion

The members of the Committee taking part in the review gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

Approved documents

The documents reviewed and approved at the meeting were:

Document	Version	Date
Notice of Substantial Amendment (non-CTIMP)	Substantial Amendment 5, 20/11/2017	17 January 2018
Participant consent form [Thank you letter]	1	05 October 2017
Participant consent form [Assent Form (Patient less than 16 years of age)]	7, highlighted	20 November 2017
Participant consent form [Informed Consent Form (Patient aged 16yrs and over)]	7, highlighted	20 November 2017
Participant consent form [Informed Consent Form (Parent)]	7, highlighted	20 November 2017
Participant information sheet (PIS) [Patient information sheet for patients aged 10-12 years]	7	17 January 2018
Participant information sheet (PIS) [Patient information sheet for patients aged 13-15 years]	7	17 January 2018
Participant information sheet (PIS) [Patient information sheet for patients aged 16+ years]	7	17 January 2018
Participant information sheet (PIS) [Parent information sheet]	8	17 January 2018
Research protocol or project proposal [Protocol]	5, highlighted	02 October 2017

Membership of the Committee

The members of the Committee who took part in the review are listed on the attached sheet.

Working with NHS Care Organisations

Sponsors should ensure that they notify the R&D office for the relevant NHS care organisation of this amendment in line with the terms detailed in the categorisation email issued by the lead nation for the study.

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16/YH/0206: Please quote this number on all correspondence
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Yours sincerely
Pp



Professor Basil Sharrack
Chair

E-mail: nrescommittee.yorkandhumber-sheffield@nhs.net

Enclosures: List of names and professions of members who took part in the review

Copy to: Ms Anne Gowing, Leeds Teaching Hospitals NHS Trust

Dr Nadia Laila Amin, University of Leeds

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