“Laboratory marker profile of patients with Juvenile Idiopathic Arthritis in a paediatric rheumatology outpatient clinic service at Tygerberg Hospital.”

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DECLARATION

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the authorship owner thereof (unless to the extent explicitly otherwise stated) and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Abstract:

Background:
Juvenile idiopathic arthritis (JIA) is the most commonly occurring chronic rheumatic disease of childhood. The diagnosis is currently a clinical one with Rheumatoid factor and HLA-B27 antigen as the only laboratory markers included in the International League of Associations for Rheumatology's classification (ILAR).

Aim:
The primary aim of the study was to examine the laboratory marker profile of patients with JIA at entry into the clinic. The secondary aim was to describe the distribution of the profile in the 7 JIA subgroups.

Method:
A retrospective, descriptive study was done at Tygerberg Hospital. It included all patients who met the diagnostic criteria (ILAR) for JIA seen at the paediatric rheumatology clinic between the clinic's inception in 1995 to July 2017. Exclusion criteria were an age older than 16 years, HIV infection or another rheumatological condition.

Results:
A total of 165 patients were recorded, with an overall predominance of female gender (58.2%), however for the subgroups of Enthesitis Related JIA, Psoriatic JIA and unspecified JIA there was a higher male to female ratio. Polyarticular JIA at 39% made up the largest subgroup, of which 15.8% were classified as poly RF positive and 23.6% as poly RF negative, closely followed by Oligoarthritis JIA, which made up 23% and which also had the youngest median age of presentation at 5 years (IQR 2-8) and 31.4% had positive antinuclear antigen (ANA). ANA positivity across the other subgroups was 23% for poly RF positive, 7.9% for poly RF negative, 8% for systemic JIA, 5% enthesitis related JIA, 100% psoriatic JIA and none in the undifferentiated group. Some studies report up to 15% of the healthy population are low grade ANA positive. [1]

Patients diagnosed with systemic JIA had significantly raised C-reactive protein (CRP) levels with a median of 150 ug/dL (IQR 95-205) with 50% having both a raised CRP and Erythrocyte sedimentation rate (ESR) with a median ESR 98 mm/hr (IQR 51-145). Furthermore 21.4% had a positive anti-streptolysin O titer (ASOT), 28% had raised Alanine aminotransferase (ALT) and all had raised platelet counts which is in keeping with the published literature of laboratory parameters indicative that systemic JIA is an autoinflammatory syndrome.

For patients with Enthesitis related JIA, 53.8% tested positive for HLA-B27 antigen. Across the 7 subgroups the ASOT was positive in 12.7% of patients which is suggestive of a background burden of Streptococcal infection in some of our cohort.

79.4% of the study cohort had a normal BMI as defined by the WHO BMI Z score charts, with 9.1% of patients classified as underweight and 4.8% classified as obese.

Conclusion:

Polyarticular JIA, as the commonest subgroup in our cohort, was Rheumatoid factor negative predominant and systemic JIA demonstrated a significant inflammatory profile, with a significantly elevated CRP (P value 0.025) and elevated platelet counts, however not statistically significant. Nonspecific ASOT elevation was seen in 12.7% of patients, most commonly in the systemic JIA subgroup, followed by poly RF negative subgroup. ANA positivity was present in all subgroups, in varying percentages, except for the undifferentiated group where all patients tested had negative ANA. 9% of patients were underweight, 9% were overweight and 4.8% were obese. As nutrition is a critical determinant of immune responses, with both micro and macronutrient deficiency altering immunocompetence and increasing risk for infection, together with the knowledge that malnutrition is the most common cause of immunodeficiency worldwide, we need to consider the impact of malnutrition on the laboratory marker profile of patients due to immune modulation of nutrient deficiency. Conversely it is appreciated that overnutrition and obesity also contribute to an altered immunity. [2,3]

Raised ASOT marker profile of our patients is suggestive of an associated Streptococcal infection burden. Baseline normal ranges of ASOT need to be established so that we may interpret the results in the context of our population where positive ranges in otherwise healthy patients may be higher. Further studies are needed to elucidate the impact of immune activation and the resultant effect on the local reference ranges of baseline autoantibodies, namely rheumatoid factor (RF) and anti-nuclear antibodies (ANA). Baseline normal ranges of ASOT need to be established so that we may interpret the results in the context of our population where positive ranges in otherwise healthy patients may well be higher.

Acknowledgements:

I would like to acknowledge and thank my supervisors Prof Monika Esser and Prof Etienne Nel. To Prof Monika Esser who assisted me in finding the topic of my dissertation, for the knowledge she imparted, for always asking about my family and how I was doing and her always friendly smile. To Prof Nel for all his support and experienced guidance and for all his assistance with the statistical analysis in the dissertation.

A special thank you to Dr Dee Abraham, who is the author of the main study this sub-study is based on, for all her advice and assistance with the data collected.

Thank you to Birhanu. T Ayele, the biostatistician at the Faculty of Epidemiology and Biostatistics and the faculty for their assistance with the statistical analysis for my dissertation.

To my two boys, Grayson and Israel, who are my greatest achievement. Who teach me daily to enjoy the simple things in life, to never be in a hurry, to sit and play, to experience the world a new for the second and third time and who forced me to learn more than I ever thought I could about dinosaurs.

To my immediate and extended family, especially my in-laws, who have been supportive along my journey and always willing to help with the boys and provide me with the quiet time I required to complete this dissertation.

Lastly, but most importantly to my husband, Malcolm, who has been my unwavering support and my rock during times of uncertainty, self-doubt and other extremes of emotion. Who always remains calm and helps to assess a situation with logic and reason and to state the facts as they are. He has been by my side since the start of my journey into medicine during medical school and then the start of my medical career. He has known and supported me in my dream to become a paediatrician and am so thankful that I can share this achievement with him.

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Introduction:

BACKGROUND AND RATIONALE:

Juvenile idiopathic arthritis (JIA) is the most commonly occurring chronic rheumatic disease of childhood and an important cause of long- and short-term disability. It is not a single disease but encompasses a diverse group of immune-mediated disorders affecting children under 16 years of age and with arthritis persisting for more than 6 weeks. It affects 1 in 1000 children worldwide. [4] The incidence and prevalence are not known in South Africa, however in 2010 a systematic review stated that the incidence varied from 1.6 to 23 per 100 000 and prevalence 3.8 to 400 per 100 000 in the populations studied which spanned across North America, Latin America, Asia, Australia, the Middle East and Europe. [4] These differences in incidence and prevalence may be explained by differences in classification used as well as methodological issues and temporal changes in incidence. The lack of previous standard diagnostic criteria between North America and Europe has complicated the ability to do epidemiological studies.

According to the International League of Associations for Rheumatology's (ILAR) classification of Juvenile Idiopathic Arthritis second revision published in 2004 [5], JIA is defined as arthritis of unknown etiology that begins before the 16th birthday and persists for at least 6 weeks with other known conditions excluded. There are 7 subgroups namely, systemic arthritis (sJIA), oligoarthritis (OligoJIA), polyarthritis rheumatoid factor negative (polyRF-), polyarthritis rheumatoid factor positive (polyRF+), psoriatic arthritis (PsJIA), enthesitis related arthritis (ERA) and undifferentiated arthritis.

The ILAR classification has standardised the nomenclature and addressed differences in European and North American criteria. The current ILAR classification assisted clinicians to communicate with a common frame of reference. However, this classification has limitations as it was based on the consensus of experts and thus some patients cannot be classified and in some there is overlap between the diagnostic categories. [6,7]

There have been concerns about unmet diagnostic needs namely, that despite a growing understanding of the disease pathogenesis, the ILAR classification is still based on the same clinical features as 20 years ago. The number of involved joints is still the hallmark for classification and determines treatment, when to escalate therapy or switch to another agent and when to taper or withdraw treatment. Division into non-overlapping categories led to an increased number of patients in the undifferentiated category. Classification systems will remain imperfect until enough is known to allow classification based on pathophysiology and genetics rather than clinical phenotypes. [6,7] Recently a new classification was proposed where international consensus is reached to identify different homogeneous chronic disorders that fall under the historical term JIA. The objective is to revise the current

ILAR criteria with an evidence-based approach, using clinical and routine laboratory measures available worldwide, to identify homogenous clinical groups. It also aims to distinguish chronic arthritis forms typically seen only in children from the childhood counterpart of adult disease. This will involve four steps, namely an initial Delphi web-based consensus, followed by an international nominal group technique (NGT) consensus conference for the new provisional Pediatric Rheumatology International Trials Organization (PRINTO) JIA classification criteria. A large database of at least 1000 new-onset JIA patients will be completed for development of evidence-based validation of the new proposed JIA classification criteria. [7]

To date the diagnosis of JIA, using the current ILAR classification, remains a clinical one, the only laboratory markers included are rheumatoid factor (RF) and Human Leukocyte Antigen B27 (HLA-B27). HLA-B27 is incorporated in the current definition either as inclusion criteria for ERA or exclusion criteria for the other subgroups. Genetic susceptibility for ERA has been proven, where HLA-B27 is positive in up to 85% of patients with ERA in the Netherlands [8] and 87% in India [9]. There is an appreciable variation in frequency of HLA-B27 antigen in different ethnicities. HLA-B27 antigen has been shown to be absent in the indigenous African population of unmixed ancestry versus prevalence of 8% of the Caucasian American population and 2-4% of the African American (assumed mixed ancestry) population. [10] Thus for diagnostic purposes the absence of HLA-B27 antigen is of less importance in excluding ERA in the African/American population versus the Caucasian population. [10]

Rheumatoid factor is also included in the classification as either an inclusion criterion for poly-RF-positive JIA or exclusion criterion for the other subgroups. In rheumatoid arthritis, rheumatoid factor can be observed years before the onset of disease, and it is still unclear if the presence of these antibodies is responsible for disease onset. Rheumatoid factor is positive in about 5-8% of all patients with JIA, where it needs to be recorded positive twice within a 3-month interval to fulfill criteria for diagnosis of poly-RF-positive JIA.

Despite only two laboratory markers included in the classification there are often multiple other laboratory markers measured during the diagnostic work up of patients with JIA. Namely, anti-nuclear antibodies (ANA), more recently Anti-cyclic citrullinated peptide antibody (anti-CCP), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), full blood count (FBC), differential count, Anti-DNaseB and Antistreptolysin O titer (ASOT), Complement 3 (C3) and complement 4 (C4), Alanine aminotransferase (ALT) as well as Extractable nuclear antigen (ENA). Of note is that the reference ranges for these laboratory markers may differ based on which laboratory method or which instrument is used at different laboratories. [11]

Different measures of diagnostic accuracy relate to the different aspects of a diagnostic test. Some measures are used to assess the discriminatory property of

the test and others are used to determine its predictive ability. The predictive measures are most useful in predicting the probability of a disease in an individual.

A perfect diagnostic test has the potential to accurately discriminate subjects with and without disease. The value of a diagnostic test is assessed by a variety of parameters, namely sensitivity, specificity, positive and negative predictive values. Values of a perfect test which are abnormal are always indicative of the disease, while the values below the cut-off limit always exclude the disease. Unfortunately, such a perfect test does not exist and therefore diagnostic tests can only make partial distinctions between subjects with and without disease.

The definition of a tests cut-off value is a critical determinant for measures of diagnostic or screening accuracy, such as sensitivity and specificity, positive and negative predictive values and correct classification accuracy. The cutoff value for a test should be selected to maximise the benefit it accumulates from testing a population. To determine the benefit of testing, misdiagnosis and prevalence in a population must be considered. There are five different methods to determine cut-off values, namely arbitrary methods, methods to optimize sensitivity and specificity, methods to optimize test accuracy, ROC curves and methods to optimize the predictive value. [12] Each one of these methods has its limitations.

Values above the cut-off are not always indicative of a disease since subjects without disease may also have elevated values. Such elevated values of certain parameters of interest are called false positive values (FP). On the other hand, values below the cut-off are mainly found in subjects without disease. However, some subjects with the disease can have levels below the cut-off too. Those values are false negative values (FN). Therefore, the cut-off value divides the population of examined subjects with and without disease in to four subgroups considering parameter values of interest:

True positive (TP) –subjects with the disease with the value of a parameter of interest above the cut-off

False positive (FP) –subjects without the disease with the value of a parameter of interest above the cut-off

True negative (TN) –subjects without the disease with the value of a parameter of interest below the cut-off

False negative (FN) –subjects with the disease with the value of a parameter of interest below the cut-off

	Subjects with disease	Subjects without disease
Positive	TP	FP
Negative	FN	TN

Sensitivity is expressed in percentage and defines the proportion of true positive subjects with the disease in a total group of subjects with the disease (TP/(TP+FN)). Sensitivity is defined as the probability of getting a positive test result in subjects with the disease. Hence, a sensitive test is most useful when negative.

Specificity is a measure that is complementary to sensitivity. It is defined as the proportion of subjects without the disease with a negative test result (TN/TN+FP). In other words, specificity represents the probability of a negative test result in a subject without the disease. Therefore, we can postulate that specificity relates to the aspect of diagnostic accuracy that describes the test ability to recognise subjects without the disease, i.e. to exclude the condition of interest.

Neither sensitivity nor specificity are influenced by the disease prevalence, meaning that results from one study could easily be transferred to some other setting with a different prevalence of the disease in the population. Nonetheless, sensitivity and specificity can vary greatly depending on the spectrum of the disease in the studied group.

Prevalence does however influence the positive and negative predictive values of diagnostic tests. As the prevalence in a population increases the positive predictive value increases and the negative predictive value decreases. Similarly, as the prevalence decreases the positive predictive value decreases and the negative predictive value increases. Where the positive predictive value is the probability that subjects with a positive screening test truly have the disease. The negative predictive value is the probability that subjects with a negative screening test truly don’t have the disease.

There is a paucity of dedicated literature on the description of laboratory markers in JIA, such as HLA-B27 positivity in ERA JIA and the inflammatory marker profile of patients with sJIA. There has been no literature for these markers within the South African context.

Diagnosis of JIA is challenging for multiple reasons in any setting and often the number of joints involved is not adequate to fulfil diagnostic criteria or patients present with arthralgia rather than arthritis. In South Africa we face additional challenges to diagnosis, namely our very diverse population demographics, access to tertiary or specialist health care, resource limitations, malnutrition and a high infectious disease burden background.

With regards to our population demographics, JIA is a disease that shows variation in expression between different ethnicities. [13] Thus, our patients may present differently to a patient in the developed world with regards to their clinical findings and laboratory markers based on differences in ethnicity.

In South Africa there is a high burden of infectious disease where two thirds of the under 5 mortality is due to diarrhea, pneumonia and human immunodeficiency virus infection (HIV). [14] A clinical association has been demonstrated for microbial

infections as triggers for autoimmune disease, where infection contributes to the initiation and exaggeration of disease. [15] Infections are considered an important environmental trigger of autoimmunity and can contribute to disease onset and severity. Infections instigate pro-inflammatory cell death programs; they induce extracellular release of host nuclear autoantigens and promote recognition in an immunogenic context by activating the innate and adaptive immune systems. [16] Rheumatoid factor, for example, has also been shown to be low-titer positive in patients with infections such as viral infections (Hepatitis B and C), infectious mononucleosis, tuberculosis, syphilis and other autoimmune diseases. [17,18]

ANA and other autoantibodies have been shown to be positive in non-autoimmune inflammatory disease, including both acute and chronic infections. Infection may be the initial step in development of an autoimmune disease and can act as an environmental primer in genetically predisposed individuals. Antibodies may be a transient phenomenon and not ultimately responsible for initiation of autoimmune disease. Molecular mimicry is a reasonable explanation for the generation of autoantibodies. Infections like EBV, Parvo B 19, Hepatitis B and C and HIV have been associated with positive ANA. [18]

South Africa has a triple disease burden of malnutrition- undernutrition, micronutrient deficiency and overweight – among children under 5 according to UNICEF's The State of the World's Children 2019. [19] 27.4% of children are stunted, 2.5% are wasted and 13% are overweight. South Africa also has the highest number of overweight children under five and adolescents between 10-19 years in the Eastern and Southern African region. Malnutrition results in varying degrees of immune modulation and this may influence some of the above-mentioned laboratory markers and the laboratory profile of our study population. [2,3] We must also consider that nutrition is a critical determinant of immune responses and that malnutrition is the most common cause of immunodeficiency worldwide. Overnutrition and obesity also reduce immunity. Micro and macronutrient deficiency alter immunocompetence and increase risk for infection. We need to consider the impact of malnutrition on the laboratory marker profile of patients due to immune modulation of nutrient deficiency. [2.3,20]

ANA is often requested as part of a general rheumatological work up in children however it is not useful as a general screening test due to its low specificity and low sensitivity with a high false positive rate. Targeted ANA should be requested when there is a suspicion of SLE or mixed connective tissue disease due to its high sensitivity or when oligoJIA is suspected as it has been shown to be an indicator of risk for uveitis. With high titers e.g.1:640, you would consider diagnoses such as SLE or MCTD and then would also request anti-dsDNA and ENA tests as there is a strong correlation. Association between positive ANA, usually of lower titer, and infection, malignancy and certain drugs has been shown. Thus, in our population

where there is a high infectious disease burden a positive ANA needs to be interpreted in context. [21]

The diagnostic utility of the ANA test is limited for multiple reasons. Firstly, the fact that a percentage (15-30%) [1,21] of healthy children have low titer positive tests and that the positive ANA results needs to be interpreted in a clinical context, depending on what diagnosis is suspected. In patients with SLE, ANA high titer positivity has a high positive predictive value and ANA with low titer has a good negative predictive value. [22] History and physical examination are necessary to determine whether an ANA test interpreted by a laboratory is diagnostically significant in a child.

There are different methods of testing for ANA and this further complicates the interpretation and comparison of values obtained. If test for ANA and positive you then need to do additional testing to characterize antigen specificity. Laboratories also vary in their standard for “positive” ANA some use titer more 1:40 and others 1:160. There are three different testing types for ANA:

1. Indirect immunofluorescence method (Fluorescent Antinuclear Antibody test): results will give you a Titer and pattern
2. Enzyme immunoassay: will give you a titer (numerical value)
3. Multiplex immunoassay

Immunofluorescence is considered the most sensitive (>95%) and gold standard for SLE but the specificity is a limitation.

In South Africa where resources are limited, where the burden of infectious diseases, poverty and malnutrition are high and socio-economic disparity is apparent, rheumatological disorders are not receiving priority for healthcare allocation. However, the morbidity, disability and effect on the quality of life associated especially with JIA and onset in early life is significant. [23]

In this study we aimed to describe the laboratory marker profile of patients with JIA at presentation to a specialist rheumatology clinic at a tertiary centre in South Africa and the potential use of these markers in aiding with diagnosis. We also describe the distribution of the 7 subgroups in our population in relation to these markers.

METHODS:

Aims:

1. To describe the serum laboratory markers in patients with Juvenile Idiopathic Arthritis at their initial visit to a paediatric rheumatology outpatient clinic service at Tygerberg Hospital.
2. To describe the different categories of Juvenile Idiopathic Arthritis within a South African context which will provide profiling of JIA within our context.
3. To determine the cost of laboratory markers with initial diagnosis.

Objectives:

1. To describe the following specific initial variables in children with Juvenile Idiopathic Arthritis:
    a. Rheumatoid factor (RF),
    b. Anti-nuclear antibodies (ANA),
    c. Anti-cyclic citrullinated peptide (anti-CCP),
    d. Erythrocyte sedimentation rate (ESR),
    e. C-reactive protein (CRP),
    f. HLA-B27
    g. White cell count (WCC), haemoglobin (Hb) and platelet count (plt)
    h. Anti-DNaseB
    i. Antistreptolysin O titer (ASOT)
    j. Double stranded DNA,
    k. Complement 3 (C3) and complement 4 (C4)
    l. Extractable nuclear antigen (ENA)
    m. Alanine aminotransferase (ALT)
2. To describe the different categories of Juvenile Idiopathic Arthritis in the sample of children attending the rheumatology clinic at Tygerberg Hospital.
3. To determine the cost spent on these laboratory markers per patient at their first visit.

Study design:

The descriptive, retrospective study was done at Tygerberg Hospital in Cape Town, which is a tertiary hospital linked to the University of Stellenbosch. It was a sub-study of a study done by Dr D Abraham entitled “The need for dedicated paediatric rheumatology services: Retrospective review of a clinic service at Tygerberg Hospital, Cape Town, South Africa.”

Study population:

The immediate drainage areas for Tygerberg Hospital are patients who reside in the following residential areas within the northern suburbs of Cape Town namely, Bellvenie, Belhar, Bellville South, Bishop Lavis, Clarkes, Connaught Estate, Cravenby, Elnor, Elsies River, Epping, Eureka, Florida, Klipkop, The Range, Uitsig and Valhalla.

Sample:

The study included all patients who met the diagnostic criteria for JIA, according to the ILAR guidelines (revised 2004) [2] seen at the paediatric rheumatology clinic. The patients that were included were seen over a period of 22 years, from the clinic's inception in 1995 until July 2017. The initial study population included 167 patients; 2 patients were excluded as they had HIV associated arthritis which left a total of 165 patients.

Variables:
The variables recorded included clinical data retrieved from medical records: patient sex, ethnicity, date of birth, age at presentation, weight, height, diagnosis and laboratory marker results collected from the National Health Laboratory System.

The body mass index was calculated using BMI = (weight in kilograms/height in meters squared). The BMI was then plotted on the WHO BMI Z score charts which are age and sex adjusted to determine whether the patients were underweight (plotted below the - 2 Z score), severely underweight (plotted below the -3 Z score), well nourished (plotted below the 1 Z score and above the -2 Z score), overweight (plotted above the +1 Z score) and obese (plotted above the +2 Z score). See Appendix 5

	Instrument and Technique used	**Reference range**
Rheumatoid factor (until April 2017)	Beckman Immage 800 Nephelometer (IgG)	0-11 IU/ml
Rheumatoid factor (after April 2017)	Roche Cobas (IgM)	0-11 IU/ml
Anti-nuclear antibody	ELISA/IFA PHD workstation, followed by reading on a fluorescent microscope	1:40
Anti-CCP*	PHD EIA workstation, the technique used was the ELISA	<20 U/ml
HLA-B27	FacsCalibur Flow Cytometer and the technique used was flow Cytometry	Negative/positive
Anti-DNaseB	Beckman-Coulter Immage and the technique used was nephelometry	Age <12 years 0-75 IU/ml

Anti-DNaseB	Beckman-Coulter Immage and the technique used was nephelometry	Age >12 years 0-200 IU/ml
ASOT*	Beckman-Coulter using the technique of nephelometry	newborns being 0-100 IU/ml, 6-12 years 0-250 IU/ml, > 12 years being 0-200 IU/ml
Double stranded DNA	BioRad, PhD ELISA workstation	negative if 0-25 IU/ml, equivocal if 26-30 IU/ml and positive if >30 IU/ml
Complement 3	Immunoturbidimetry with Roche Cobus analyser	0.9-1.8 g/L
Complement 4	Immunoturbidimetry with Roche Cobus analyser	0.9-1.8 g/L
ENA*	BioRad, PhD ELISA workstation	negative if 0-19.9 IU/ml, equivocal 20-24.9 IU/ml and positive if >25 IU/ml
ESR*	manual method with Westergren technique	normal reference range of 0-10 mm/hr
CRP*	Beckman-Coulter using nephelometry	0-8 ug/dl
	Roche Cobas analyser using either chemiluminescence or turbimetry	0-8 ug/dl
Haemoglobin	Advia 20-120 and technique used was spectrophotometry	*Appendix 1
White cell count	Advia 2120i haematology system with autoslide and the technique used was spectrophotometry	*Appendix 2
Platelet count	manual counting on a cell counter	*Appendix 3
ALT* (before Sept 2016)	Siemens, modified IFCC	5-30 U/L
ALT (After sept 2016)	the Roche, modified IFCC	5-30 U/L

*Anti-CCP: Anti- Cyclic citrullinated peptide antibody, ASOT: Anti-streptolysin O Titer, ENA: Extractable Nuclear Antigen, ESR: Erythrocyte Sedimentation Rate, CRP: C-reactive protein, ALT: Alanine aminotransferase

All the above-mentioned laboratory markers were measured in an accredited, certified laboratory, namely the NHLS at Tygerberg Hospital.

DATA MANAGEMENT AND STATISTICAL ANALYSIS:

Capture and security:

Microsoft Excel was used to capture all the data. The Excel spreadsheets were password protected and stored on devices that were also password protected.

Cleaning:

A total of 167 patients were captured however 2 patients excluded as they were diagnosed with HIV-associated arthritis.

Statistical analysis:

All statistical analyses were carried out using Stata 14.

The sample characteristics which were categorical in nature were described using frequencies and percentages. Continuous variables such as age as well as all the laboratory markers were summarized using median and interquartile range as they were not normally distributed.

Categorical data was compared using the Chi test for trend and quantitative comparisons used the Kruskal-Wallis test. Bonferroni adjustment was applied as a correction method for multiple comparisons to explore the post-hoc differences between pairs of patient groups. All statistical tests were two-sided and p values <0.05 were considered significant.

ETHICAL CONSIDERATIONS:

The study was approved by the Health Research Ethics Committee of the Faculty of Health Sciences of the University of Stellenbosch (protocol number N16/03/039A) and the Research Committee of the Department of Medicine, Tygerberg Hospital. The study was conducted in accordance with Medical Research Council and ICH guidelines.

Patients were assigned study codes and identifying details were not used on the data capture sheets, thus ensuring confidentiality. Microsoft Excel was used to capture the data.

As the study design was a retrospective, descriptive study the risks were minimal, and no contact was made with patients. Patients were not subjected to any further testing as already existing data was used. A waiver of individual informed consent was requested from the Health Research Ethics Committee as this is a retrospective folder review and is a sub study of another study.

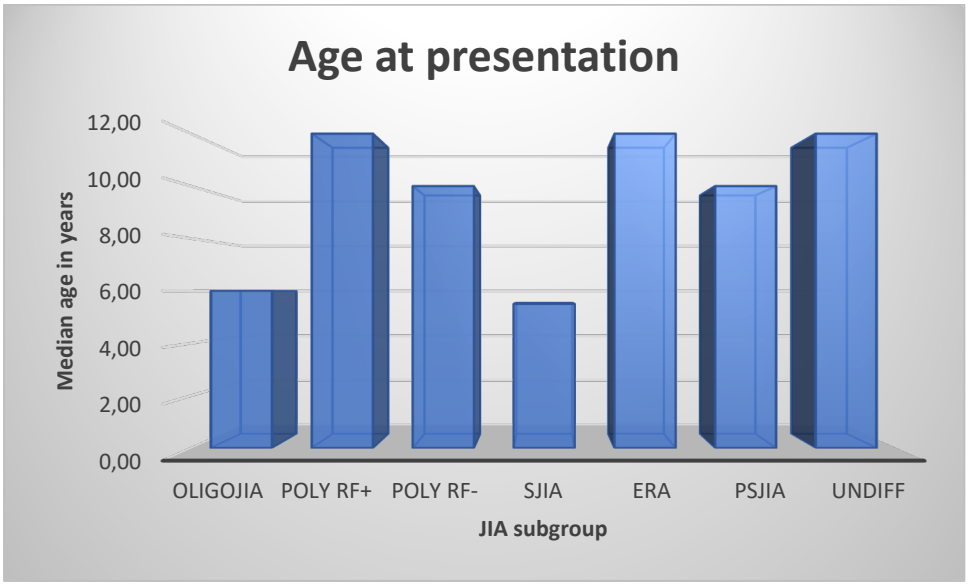
Results:

Patient characteristics:

The study comprised a total of one hundred and sixty-five patients with the diagnosis of Juvenile Idiopathic Arthritis during the study period of 1995 until July 2017.

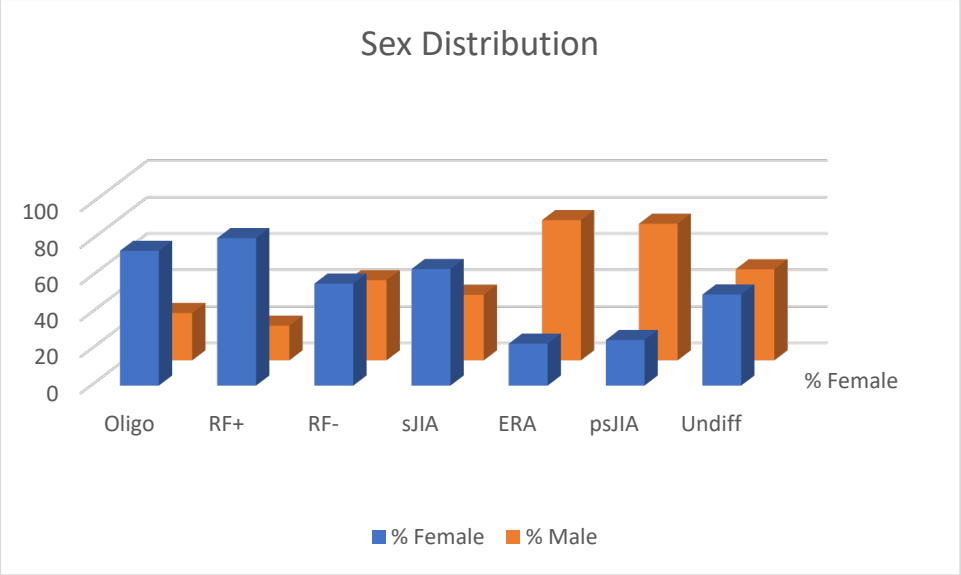
Age:

The age at youngest presentation was 1 year old and the oldest was 16 years old. The median age at presentation for both males and females were 10 years (IQR 3.8-16). The youngest age at presentation per subgroup was Oligo JIA at 5 years (IQR 2-8) followed by systemic JIA at 5.5 years (1.7-9.3).



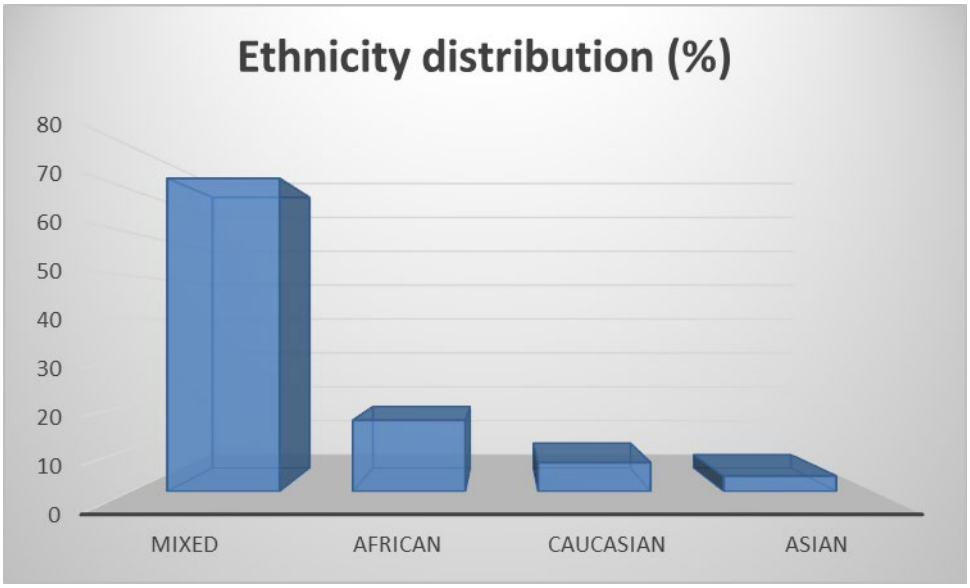
Sex:

Most of the study population was female (58.2%), with a higher female to male ratio in the subgroups Oligo JIA, both RF positive and negative subgroups and sJIA. For the subgroups ERA JIA, psJIA and undiff JIA there was a higher male to female ratio. This is in keeping with the current literature.



Ethnicity:

Majority of the study patients were of mixed ancestry (72.6%), followed by African (16.5%), Caucasian (6.7%), Asian (3.6%). In one patient no race was captured.

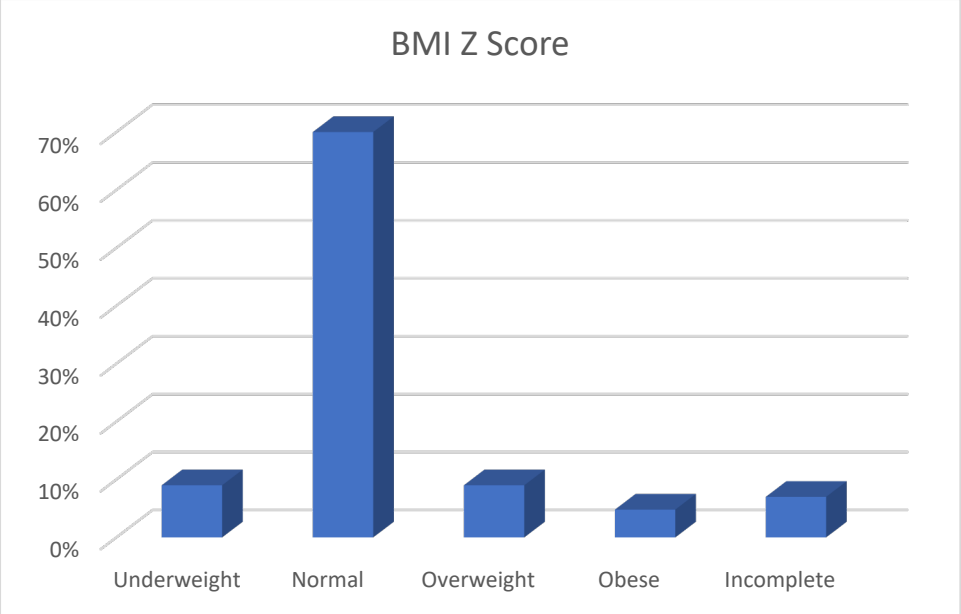


Nutritional status:

The World Health Organisation BMI Z-score charts were used to determine the nutritional status of the study population. See Appendix 1

A total of 116, which made up 70% of the study sample had a normal BMI plotting between the +1 Z score and the -1 Z score for age.

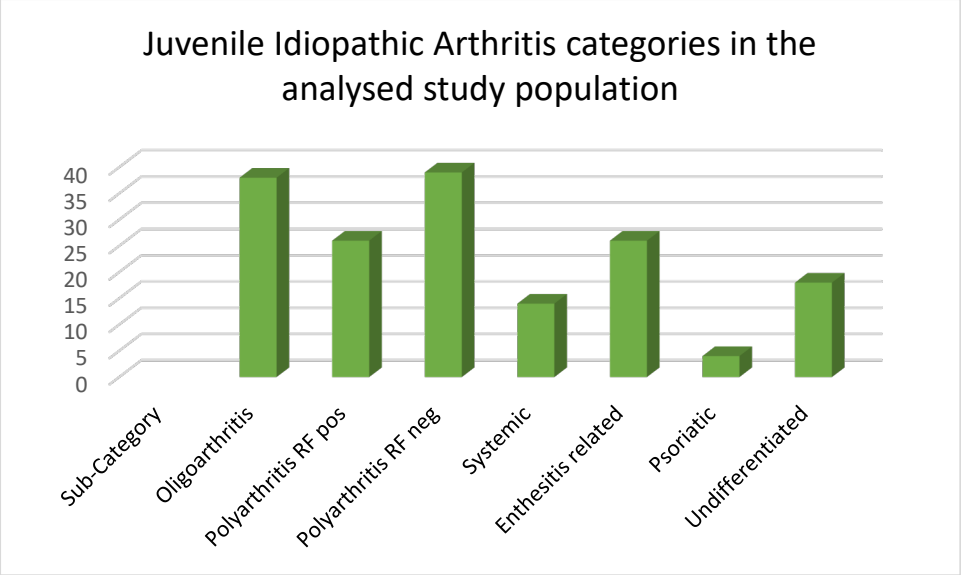
Fifteen patients (9%) were classified as underweight with a BMI below the -2 Z score. Of these, two had Oligo JIA, four poly RF negative JIA, three poly RF positive JIA, two systemic JIA, two ERA JIA, one psoriatic and one undifferentiated JIA.
Fifteen patients (9%) were classified as overweight (BMI Z score >+1) and eight patients (5%) were classified as obese (BMI score above +2 Z score). Of which three were diagnosed with Oligo JIA, one with poly RF negative JIA, one systemic JIA and two with undifferentiated JIA.
Eleven patients (7%) could not be classified due to incomplete data.



JIA subgroups:

The largest group of patients were in the PolyRF- subgroup (23.6%), closely followed by 23% in the OligoJIA subgroup.

Juvenile Idiopathic Arthritis categories in our South African cohort	
JIA categories	Study population [N=165]
	N (%)
Oligoarthritis	38 (23)
Polyarticular RF positive	26 (15.8)
Polyarticular RF negative	39 (23.6)
Systemic	14 (8.5)
Enthesitis related	26 (15.8)
Psoriatic	4 (2.4)
Undifferentiated	18 (10.9)



Oligoarthritis Juvenile Idiopathic Arthritis:

This subgroup comprised a total of thirty-eight patients, making up 23% of the study population. The median age at presentation was 5 years old (IQR 2-8), which was the youngest presentation across all 7 subgroups. Majority of the patients were female (73.7%)

For laboratory marker profile, 35 patients had ANA tested and 11 were positive (31.4%). Of those ANA positive the respective titers were 36.4% (1:40), 36.4% (1:160), 27.2% (1:320). Sixty eight percent (68%) of patients had an elevated ESR, with a median value of 31.5 mm/hr. (IQR 16.5-46.5) Forty six percent (46%) of those tested had a raised CRP with a median value of 57 ug/dl (IQR 44.7-69.2) Where four patients had both a positive ESR and CRP. Twenty-one (21%) had a positive ASOT with a median value of 158 IU/ml (IQR 78-238) and of the 18 patients tested for Anti-DNaseB, 11 (61%) were positive indicating current or previous streptococcal infection. One patient tested positive for rheumatoid factor. All patients tested were HLA-B27 and anti-dsDNA negative.

The median ALT was 18 U/L (IQR 13-23), only one patient had a raised ALT. The median white cell count (WCC) was 10 x 10^9/l (IQR 1-19) with three patients having raised counts for their age, the median Hb was 12 g/dl (IQR 11.7-12.3) and one patient had a low Hb and the median plt count was 383 x10^9/l (IQR 292-473) of which 10 patients had raised counts.

For Polyarthritis Rheumatoid Factor positive:

This subgroup comprised a total of twenty-six patients, making up 15.8%. The median age at presentation was 12 years old (IQR 9-15). Majority of the patients were female (81%).

For laboratory marker profile, all patients had a positive rheumatoid factor. However not every patient had two RF done, three months apart as per the ILAR classification due to cost constraints. Twenty three percent (23%) of patients had a positive ANA. Of those ANA positive the respective titers were 17% positive (1:40), 66% (1:160), 17% (1:320). The ASOT was positive in 14.2% of this group with a median value of 134 IU/ml (IQR 113-156) and twenty nine percent (29%) of patients were Anti-DNaseB positive indicating current or previous streptococcal infection. A total of 8 patients were tested for anti-dsDNA and only 2 of the 8 were positive. Anti-dsDNA is usually only requested if ANA is positive and when a diagnosis of SLE is suspected as it is highly specific. In these patients the test served as confirmation for the diagnosis of SLE as an overlap diagnosis A total of 22 patients were tested for anti-CCP, 14 tested positive (63.6%).

With regards to the inflammatory markers, seventy eight percent (78%) had raised ESR's with a median of 40 mm/hr (IQR 18.5-61.5) and 6% had a raised CRP, with a median of 14 ug/dl. (IQR 8.9-17.2) Where only 2 patients had both a positive ESR and CRP.

The median ALT was 21 U/L with 3 patients having raised level (IQR 14.5-27.5). The median WCC was 9 x10^9/l with 4 patients having raised counts (IQR 7.5-11.5). The median Hb 12 g/dl with 5 patients with a low count (IQR 11-13). The median plt were 374 x 10^9/l with 7 patients having a raised count (IQR 311-437).

For Polyarthritis Rheumatoid Factor negative:

This subgroup comprised a total of thirty-nine patients, making up 23.6 %. The median age at presentation was 10 years old (IQR 4.5-15.5). Majority of the patients (56%) were female.

For the laboratory marker profile, all the patients had a negative rheumatoid factor except for three and two of those three had a repeat rheumatoid factor that was negative, and one patient had only one test done with a very low titer. As per the ILAR classification you need to have two positive tests three months apart to be classified into the poly RF positive group thus these patients were classified as poly RF negative.

All patients except one had ANA titers done and 15% of those tested had positive ANA's. Of those with positive ANA the respective titers were 50 % (1:40), 33% (1:160) and 17% (1:320). Of the 21 patients tested for anti-CCP all were negative. RF negativity was a good predictor of anti-CCP negativity in our study. All patients tested for ENA were negative. Eighteen percent (18%) of patients tested were HLA-B 27 positive, only 8 patients tested for anti-dsDNA with one positive. Fifty percent (50%) had a positive ASOT with median value 551 IU/ml (IQR 354-748) which was the highest median across all 7 subgroups but was not statistically significant with a p value of 0.12 and 74% tested positive for Anti-DNaseB indicating current or previous streptococcal infection. Even though not statistically significant, a positive

ASOT or Anti-DNaseB may serve as an alert to exclude intercurrent or untreated streptococcal infection as a driver for the autoimmune disease.

With regards to the inflammatory markers, 77% had an elevated ESR with median value of 49 mm/hr (IQR 19-80) and 44% had elevated CRP's with median value of 58 ug/dl. (IQR 33-83) where only 2 patients had both positive ESR and CRP.

The median ALT was 16 U/L, with only two patients having raised ALT levels (IQR 11.5-20.5). The median WCC was 8 x10^9/l, with only one patient having a raised count (IQR 6-10). The median Hb was 12 g/dl, with 5 patients having low Hb (IQR 11-13). The median plt count was 427 x10^9/l with 19 patients having raised count. (IQR 337-517)

For Systemic JIA:

This subgroup comprised 14 patients which makes up 8.5% of the total. The median age at presentation was 5.5 years (IQR 1.7-9.3), the second youngest across all subgroups. Most patients were female (64%).

For the laboratory marker profile, only one patient had a positive ANA, with titer of 1:40. All those that were tested for rheumatoid factor, HLA-B27, anti-dsDNA, ENA were negative. For anti-CCP one patient tested positive. For ASOT 60% of patients tested were positive with a median titer of 241 IU/ml (IQR 208.5-273) and two of the four patients tested for Anti-DNaseB were positive indicating current or previous streptococcal infection. Even though not statistically significant, a positive ASOT or Anti-DNaseB may serve as an alert to exclude intercurrent or untreated streptococcal infection as a driver for the autoimmune disease. One patient had elevated levels of C3 and C4.

For the inflammatory markers 70% tested had an elevated ESR and 100% tested had positive CRP's with median values respectively of 98 mm/hr (IQR 50.7-97) and 150 ug/dl (IQR 94.8-155.2). The median CRP which was elevated was statistically significant, with a p value 0.025 in comparison to the median CRP across the other 6 subgroups. The median ESR was also the highest across all 7 subgroups but was not statistically significant with a p value of 0.188.

The median ALT was 21 U/L (IQR 7.8-34.3) with 28% of patients having a raised ALT level. The median WCC was 18 x10^9/l (IQR 4.75-31.25) with 6 patients having a raised WCC. The median Hb was 9 g/dl (IQR 7.75- 10.25) with 5 having low counts. The median plt count was 638 x10^9/l (IQR 512-764) with all patients tested having a raised count (9 patients).

For enthesitis related JIA:

This subgroup comprised 26 patients which makes up 15.8% of the total. The median age at presentation was 12 years (IQR 10.7-13.2) with a male predominance, with 77% of patients being male.

The laboratory marker profile showed that only one patient of those tested had a positive ANA with a titer 1:640. Of those tested for rheumatoid factor 11.5% tested positive. All patients tested for anti-CCP were negative as well as for ENA, anti-dsDNA, C3 and C4. 55% tested positive for Anti-DNaseB and one patient had a positive ASOT, indicating underlying or previous streptococcal infection. 58% tested positive for HLA-B27.

With regards to the inflammatory markers, 73% had an elevated ESR with a median value of 50 mm/hr (IQR 25-75) and 29% had an elevated CRP with a median value of 60 ug/dl. (IQR 0-14) where three patients had both a raised ESR and CRP.

The median ALT was 16 U/L (IQR 11.5-20.5) with four patients having an elevated level. The median value measured for WCC was 9 x10^9/l (IQR 7-11) with 5 having an elevated level. The median measured for Hb was 12 g/dl (IQR 11-13) with all patients having an Hb within the normal range for age. The median plt count was 405 x10^9/l (IQR 315-495) with 10 patients having an elevated count.

For Psoriatic JIA:

This subgroup was comprised of only 4 patients, making up 2.4% of the study population.

For ANA, only 2 patients were tested, and both had positive ANA with titers of 1:40 and 1:160 respectively. Those that were tested all had negative rheumatoid factors, anti-CCP, HLA-B27 and ASOT. Some laboratory markers were not tested at all for this sub-group, namely anti-dsDNA, ENA, Anti-DNaseB, C3 and C4.

With regards to inflammatory markers all patients were tested for ESR and 50% had an elevated value with a median value 49 mm/hr (IQR 33.5-64.5) None were tested for CRP.

The median ALT was 13U/L (IQR 12.5-13.5) with all the values being within the normal range. The median WCC 8 x10^9/l (IQR 7.5-8.5) with all values normal for the age. The median Hb 13 g/dl (IQR 12-14) with one patient having a low count. The median plt count was 279 x10^9/l (IQR 254-305) with all patients having normal counts.

For undifferentiated JIA:

This subgroup comprised 18 patients which made up 10.9% of the study population.

For ANA, all patients that were tested, which comprised 16 patients, were ANA negative. For rheumatoid factor 13.3% tested positive (15 patients tested). Of those tested all had negative anti-CCP, anti-dsDNA, ENA, C3 and C4. Of the 5 patients tested for Anti-DNaseB, 60% were positive and one patient tested positive for ASOT indicating current or previous streptococcal infection. Of those 10 tested for HLA-B27, 40% positive. These laboratory findings demonstrate that due to limitations of the

current classification some of the patients in this subgroup with features of JIA may still be evolving into a classical diagnosis.

With regards to inflammatory markers, 14 patients had ESR tested and 50% positive with a median value of 25 mm/hr (IQR 15-35). And of the 3 patients tested for CRP, one tested positive with a value of 11 ug/dl. This patient also had a positive ESR.

The median ALT was 21 U/L (IQR 16.5-25.5) and no patients had an elevated level. The median WCC was 7 x10^9/l (IQR 6-8) with one patient having an elevated count. The median for Hb was 13 g/dl (IQR 12-14) with one patient having a low count. The median plt count was 331 x 10^9/l (IQR 297-365) with 3 patients having an elevated count.

General remarks on testing:

For our study population, 68 of 168 were screened for HIV of which 3 were positive and thus excluded from the study. Ninety-three (93) patients were screened for tuberculosis (TB), of which 12 were positive and received treatment based on either clinical, radiological and/or microbiological evidence.

Routine measurement of Anti-CCP was only instituted in 2012 in patients with polyarticular JIA. For the poly RF positive subgroup 22 patients had anti-CCP testing done of which 14 were positive (63.6%). For the poly RF negative subgroup 21 were tested and all had negative anti-CCP. RF negativity was a good predictor of anti-CCP negativity in our study. Patients with a positive anti-CCP have been shown to progress to the adult type rheumatoid arthritis.

82.4% of the study population had an ESR measured, of which 72.8% had an elevated value. Only 27.7% had a CRP measured, of which 52.3% were elevated, with only 10.3% having had both an ESR and CRP measured.

Table 1:

Number of tests done across JIA sub-categories							
	OligoJIA [N=38]	PolyRF+ [N=26]	PolyRF- [N=39]	Systemic [N=14]	ERA [N=26]	Psoriatic [N=4]	Undiffer [N=18]
Full blood count	30	21	35	9	22	3	14
Full blood count and ALT	25	19	31	5	21	2	11
ASOT and anti-DnaseB	17	7	19	4	11	0	5
ESR and CRP	8	5	7	6	7	0	3
RF and ANA	32	26	38	12	22	2	15
HLA-B27 antigen	11	16	17	5	24	3	10

Table 2:

Laboratory marker profile across JIA sub-categories							
	OligoJIA [N=38]	**PolyRF+ [N=26]**	**PolyRF- [N=39]**	**Systemic [N=14]**	**ERA [N=26]**	**Psoriatic [N=4]**	**Undiffer [N=18]**
RF [number of seropositive/tested] (%)	1/32 (3%)	26/26 (100%)	3/39 (7.7%)	0/12	3/23 (13%)	0/3	2/15 (13.35%)
ANA [number of seropositive/tested] (%)	11/35 (31.4%)	6/26 (23%)	6/38 (7.9%)	1/12 (8%)	1/22 (5%)	2/2 (100%)	0/16
ASOT [number of seropositive/tested] (%)	4/19 (21%)	1/7 (14.2%)	11/22 (50%)	3/5 (60%)	1/11 (9%)	0/1	1/7 (14%)
HLA-B27 [number of seropositive/tested] (%)	0/11	1/16 (6%)	3/17 (18%)	0/5	14/24 (58%)	0/3	4/10 (40%)
Anti-DNAsB [number of seropositive/tested] (%)	11/18 (61%)	2/7 (29%)	14/19 (74%)	2/4 (50%)	6/11 (55%)		3/5 (60%)
DsDNA [number of seropositive/tested] (%)	0/8	2/8 (25%)	1/8 (13%)	0/3	0/2		0/4
Anti-CCP [number of seropositive/tested] (%)	0/12	14/22 (64%)	0/11	1/5 (20%)	0/12	0/1	0/5
CRP (ug/dl) median	6/13 (46%) 57	3/5 (6%) 14	4/9 (44%) 58	7/7 (100%) **150**	2/7 (29%) 60	0	1/3 (33%) 11
ESR (mm/hr) median	19/28 (68%) 28	18/23 (78%) 40	27/35 (77%) 49	7/10 (70%) 79	16/22 (73%) 50	2/4 (50%) 49	7/14 (50%) 25.0
Haemoglobin (g/dl)	12	12	12	9	12	13	13
White cell count (10^9/l)	10	9	8	18	9	8	7.0
Platelet (10^9/l)	383	374	427	638	405	279	331.0

Discussion:

Demographics of our study sample, from the Western Cape, reflect the diverse ethnicity within South Africa. Stats SA (2011) from the census [24] stated that the majority of the Western Cape population was of mixed ancestry (48.8%), followed by African (32.8%), then Caucasian (15.7%) and lastly Indian/Asian (1%) and other (1.6%) Worldwide there are geographical differences in the epidemiology of JIA based on ethnic differences with incidence rates that very from 1.6 – 23 per 100 000 and prevalence rates from 16– 150 per 100 000. [25] We were unfortunately not able to calculate the prevalence for our study population.

In a multiethnic cohort from Toronto, Canada, those children from European descent had a significantly increased risk (Risk ratio of 1.26 with P value =0.005) of developing JIA in comparison to those of non-European descent (Risk ratio of 0.43). [26] The incidence in Oman was 2 per 100 000 and prevalence 20 per 100 000. [27] In France the incidence was 3.2 per 100 000 and prevalence 19.8 per 100 000. [28] Our study population included patients from different ethnicities as mentioned earlier where most patients were of mixed ancestry (72.6%), followed by African (16.5%), Caucasian (6.7%) and Asian (3.6%).

These differences in reported incidence may also be attributable to changes in classification, changes in temporal incidence rather than real differences or be linked to the type of study done such as a clinic-based versus population-based study and case ascertainment methods which may vary between accurate systematic health visits attended and survey-based methods or questionnaires.

There is also significant geographic variation in the epidemiology of JIA. According to the distribution within our study population, the most frequent subgroup of JIA diagnosed was poly RF- JIA (23.6%), closely followed by OligoJIA (23%). In a study done in the Western Cape, South Africa [29] based in two tertiary centers both poly RF- and OligoJIA were equally prevalent closely followed by ERA JIA, which is consistent with our study. Our distribution of subgroups is in keeping with studies published in other developing countries such as Oman and Zambia [27,30] where poly RF- JIA is predominant followed by OligoJIA compared to developed countries where OligoJIA is the most prevalent subgroup followed by poly RF- JIA such as Poland, Turkey and France. [31,32,28]

Antinuclear antibodies (ANA) are antibodies directed against a range of different nuclear constituents and other cellular components. They are found in the serum of patients with auto-immune disease (e.g. oligoarticular JIA, systemic lupus erythematosus and Sjogren's syndrome). Although ANA's are present in 15% of healthy children (with 9% at serum dilution 1:40, 3% at 1:80 and 3% in 1:160) and thus cannot be used as a diagnostic tool per se, there is a close relationship between ANA positivity in JIA and a younger age at disease presentation, female predominance, asymmetric arthritis, development of uveitis, lower number of affected joints over time and a lack of hip involvement. [1]

When interpreting ANA results, we would also need to consider the possibility of a high infection burden within our study population and that our "healthy" population may have higher ANA baseline positivity than previously reported 15%.

The diagnostic utility of the antinuclear antigen test is limited due to the large number of healthy children who have low-titer positive tests [1] as well as the effect of acute and chronic infection on the test. [18] So, when ANA is used as an initial screening test in patients with non-specific clinical symptoms, the likelihood is higher of a false positive result. [38]. Anti-dsDNA antibodies however have greater specificity for autoimmune diseases like e.g. SLE. In our cohort however this marker, anti-dsDNA, was also seen in polyarticular JIA group, which could possibly indicate that a subgroup of these patients evolved later into reclassification of SLE. [46]

There is an appreciable variation in frequency of HLA-B27 antigen in different ethnicities. HLA-B27 antigen has been shown to be absent in the African Black population of unmixed ancestry versus present in 8% of the Caucasian American population and 2-4% of the American Black (assumed mixed ancestry) population. [10] Thus for diagnostic purposes the absence of HLA-B27 antigen is of less importance in excluding ERA in the African/American black population versus the Caucasian population. [10] An older male child with joint symptoms and who is HLA-B27 antigen positive is likely to fall into the ERA subgroup and receive targeted treatment.

CRP and ESR, which are both inflammatory parameters, are two biomarkers related to disease activity. CRP is an acute phase protein, produced by the liver in increased amounts during inflammation. The origin of the inflammation is not related to the increase in CRP; thus, it cannot be used to distinguish between infective cause of arthritis and sterile JIA. ESR is another general marker of inflammation, its increase occurs later during inflammation and can still be present when the inflammation is already under control. Therefore, it has less value for acute situations.

The American College of Rheumatology developed consensus guidelines for JIA treatment in 2011 which incorporates CRP and ESR. These markers are included as features of poor prognosis for the subgroups oligoJIA, PsJIA, ERA JIA, the undifferentiated and sJIA. CRP and ESR, when raised, are considered a feature of higher disease activity. [1,41]

PolyRF- JIA typically has a biphasic age of onset with the early peak between 1 and 4 years and the later peak between 6 and 12 years.[33] For the poly RF- subgroup, which made up the largest group in our study (23.6%), the median age of onset was 9 years, demonstrating a later peak in our study population. 74% of patients presented after their 5th birthday, with a sex ratio (F:M) of 1:1. All patients tested negative for rheumatoid factor and all that had anti-CCP's tested were negative. Of note is that anti-CCP testing was only introduced in 2012. In our cohort a negative RF seemed to be a good predictor for a negative anti-CCP. Where Rheumatoid Factor is negative, but the patient has a positive anti-CCP, it is indicative of an emerging adult type of polyarticular rheumatoid arthritis where the patient is often sicker, has a poorer outcome and more aggressive disease. [1]

Rheumatoid factor positivity can be observed before the onset of clinical disease. Rheumatoid Factor is positive in about 5-8% of patients with JIA [1] and is used for classification of polyarticular JIA. This subgroup is very similar to adult rheumatoid arthritis, featuring progressive symmetrical arthritis of the small joints in the hands and feet with tendency of joint erosions. RF is both a diagnostic and prognostic marker in children with JIA since it is highly specific and reflects a subgroup of JIA patients with a marked clinical picture and worse outcome. [1]

Anti-CCP antibodies are auto-antibodies directed against citrullinated protein antigens. Arginine residues in proteins can be post-translationally modified and changed into a citrulline residue. These citrullinated proteins are targeted by anti-CCP antibodies, resulting in formation of immune complexes. It has been shown that anti-CCP positivity and bony erosions, the degree of joint damage, and ESR levels were significantly correlated. [34] Anti-CCP when positive predicts which patients will go on to develop adult type rheumatoid arthritis. A meta-analysis of anti-CCP in JIA showed a pooled sensitivity of only 12% in JIA, but the good diagnostic accuracy of this assay was mainly due to its specificity 99%. Therefore, a positive ant-CCP antibody supports the diagnosis of JIA but a negative test does not exclude it. [1,35]

Anti-cyclic citrullinated peptide antibody (anti-CCP) has been included in the American College of Rheumatology's adult criteria for Rheumatoid arthritis classification. [36]

A clinical association has been demonstrated between microbial infection and rheumatoid arthritis, where infection contributes to the initiation and exaggeration of rheumatoid arthritis. [16,17] Rheumatoid factor has also been shown to be low-titer positive in patients with infections such as Hepatitis B and C, infectious mononucleosis, tuberculosis, syphilis and HIV. Elevated levels of anti-CCP and RF have been shown to lack specificity in immune compromised, i.e. HIV positive individuals, and normalization on immune reconstitution. [37]

Of note is that for the poly RF – subgroup there was high rate of positive ASOT, 50%, and 74% positive for Anti-DNaseB together with a higher ESR and CRP that could indicate a microbial trigger for this subgroup.

The second commonest subgroup in our study was OligoJIA, making up 23% of the study cohort. The age at onset distribution normally has a peak between 2 and 4 years with a small proportion presenting later. However, in our study population the median age of onset was older at 5 years. The sex ratio for this group was (F: M) 2.8:1 in comparison to Western countries where the sex ration is 5-6:1. For this subgroup, 29% had positive ANA titers with 7.9% having a titer 1:320.

The poly RF+ JIA subgroup made up the third largest group (15.7%). The median age was 12 years, where all patients presented after their 5th birthday. Once again, our population demonstrated a later peak of presentation. [33] (The bimodal distribution of age of onset, first peak between 2-5 years and second between 10-14 years). There was a sex ratio (F: M) of 4:1 with 81% of patient's female. All patients tested positive for RF but not all patients had a second confirmatory test done 3 months later due to resource constraints. For this subgroup, 3.8% tested positive for ASOT and 36% tested positive for Anti-DNaseB. In some of these patients this could be a false positive RF due to infection versus an epigenetic switch factor with immune activation and resultant JIA. [16]

For the ERA JIA subgroup, which comprised 15.7% of the study population, the median age of presentation was 12 years with a male predominance and sex ratio (F:M) of 1:4.3. This is in keeping with the pattern found worldwide of late age of presentation and male predominance. HLA-B27 antigen positivity remains the largest known contributor of disease hereditability. Genetic susceptibility for ERA JIA is proven with HLA-B27 antigen positive in up to 85% of patients in the Netherlands with ERA [8] and 87% in India with ERA [9], while the background rate is much lower in the healthy population for example 7.8% Netherlands. [8] Of those tested for HLA-B27 in those with ERA JIA (24 of the total 26) in our study, 53.8% were found to be positive. ANA was rarely, if ever positive in ERA subgroup.

Systemic JIA accounted for 8.5% of our study population. The median age of presentation was 5.5 years which is in keeping with the literature that these patients present earlier as they are systemically unwell. There was a slight female predominance with a sex ratio of (F: M) 1.5:1. All patients that were tested for HLA-B27, rheumatoid factor and anti-dsDNA tested negative. For the inflammatory markers, 50% of patients had both positive ESR and CRP's with median values respectively of 98 mm/hr and 150 ug/dl. The median CRP of 150 ug/dl was significantly higher than the other subgroup's, with a p value 0.025. This inflammatory profile with elevated CRP, ESR, platelets and WCC with low Hb and could be useful in a child for early classification into sJIA subgroup and correct interventive medicine for this subgroup with high morbidity and even mortality.

Many patients in the sJIA subgroup had abnormal full blood count values and ALT values, however they did not differ significantly from the other patients in our sample. The median ALT was 21 U/L with 28% of patients having a raised ALT level. The median WCC was 18 x10^9/l with 6 patients having a raised WCC. The median Hb was 9 g/dl with 5 having low counts. The median plt count was 638 x10^9/l with all patients tested having a raised count (9 patients). The above results demonstrate the auto inflammatory nature of sJIA.

Systemic JIA represents about 4-17% of total JIA cases. [1] There have been various studies that have helped decipher the immunological basis underlying the clinical heterogeneity of JIA. One of the most significant breakthroughs was the realisation that sJIA, a subgroup with strong systemic clinical symptoms, has the immunological signature of an auto inflammatory rather than a classical auto-immune disease. [40] It is clinically as well as immunologically distinct from the other subtypes. The diagnosis is heavily based on the exclusion of other disorders thus prolonging the time to final diagnosis. This increases the chance of developing macrophage activation syndrome if there is a delay in initiating treatment.

PsJIA made up 2.4% of the study population with a male predominance and sex ratio (F: M) 1:3 which is in keeping with the current literature.

The undiff JIA subgroup made up 10.9% of the study population with a sex ratio of (F: M) 1:1. All patients tested for ANA (16) were negative, for rheumatoid factor 11.1% tested positive (15 patients tested), 16.7% tested positive for Anti-DNaseB and 22.2% tested positive for HLA-B27 antigen. With regards to inflammatory markers, 38.9% had a positive ESR with a median value of 25 mm/hr and 5.6% had a positive CRP with a median value of 11 ug/dl. This subgroup especially with positive markers such as HLA-B27 deserves further follow up to establish a final JIA category.

JIA remains a diagnosis of exclusion and many patients had numerous laboratory tests done by the time they were referred to a paediatric rheumatologist as was

experienced in our study sample. (Refer to table 1) Basic laboratory markers like a full blood count together with the inflammatory markers, CRP and ESR, are commonly requested. C3, C4, and ASOT can help differentiate JIA from post-streptococcal arthritis. Other infections like TB and HIV need to be excluded not only when the diagnosis of arthritis is considered but before starting any immune modulating therapy. Once there is a suspicion of a JIA diagnosis, rheumatoid factor and ANA will usually also then be requested. For our study population 91% had had an ANA and RF done already on entry into the clinic. It is important to remember that age and clinical pattern of arthritis should guide the indications for these tests. (Refer to Table 1)

The usefulness of other laboratory markers is dependent on which clinical subgroup is suspected. Thus, if the history and examination is not suggestive of ERA JIA then HLA-B27 antigen testing is not indicated.

When interpreting laboratory tests, especially when treating patients who may have been referred in from another health facility, we also need to take in to account that different laboratories may use different methods and instruments to run the various laboratory markers mentioned above and thus may have different reference ranges.

The financial implications of laboratory testing for JIA need to be considered considering the limited value of these for making a definitive diagnosis. For our study population, at presentation, on average the cost spent on laboratory markers was R204.72, for the commonly requested laboratory markers (FBC, ALT, ESR, and CRP). The cost increased once antibody testing was added with an average cost of R 619.04. ANA cost R139 and RF R73.38, CRP R 72.96, ESR R28.32, C3 and C4 R 73.38, HIV ELISA R 55.18. The tariffs stated above are from 2019 and are state rates and may very well be more expensive for private patients on medical aids.

Table 3:

Cost of laboratory markers (average cost)							
	OligoJIA	**PolyRF+**	**PolyRF-**	**Systemic**	**ERA**	**Psoriatic**	**Undiff**
Full blood count	R1740	R630	R2262	R522	R1276	R174	R812
Full blood count and ALT	R2575	R1957	R3193	R515	R2163	R206	R1133
ASOT and anti-DnaseB	R1768	R728	R1976	R416	R1144	R0	R520
ESR and CRP	R800	R500	R700	R600	R700	R0	R300
RF and ANA	R6784	R5512	R8056	R2544	R4664	R424	R3180
HLA-B 27 antigen	R770	R1120	R1190	R350	R1680	R210	R700

**The table above is based on the numbers from table 1.

As per the general definition in the ILAR classification, JIA has unknown aetiology and other conditions need to be excluded as the cause of the arthritis. In our study the majority of the study population appears to have a high burden of infections such

as streptococcal infections evidenced by raised ASOT and Anti-DNaseB unrelated to their JIA in the preceding months prior to presentation, have a higher incidence of malnutrition, latent or active TB or are infected with HIV or HIV exposed. 13% of our study population was diagnosed with TB and all patients with HIV were excluded from the study but HIV exposure cannot be easily quantified. All the above influence various autoantibody markers as well as inflammatory markers and basic laboratory markers like FBC. [37,42] Confirmation of TB latency prior to initiating certain forms of immunosuppressive therapy such as Tissue Necrosis Factor- alpha blockers is essential. [43]

Screening for latent tuberculosis infection and/or disease, can be done by a number of tests such as the Mantoux skin test, chest X-ray, sputum or gastric washings for auramine stain, GeneXpert and TB culture or QuantiFERON gold TB test (Interferon Gamma Release Assay), whichever is the most appropriate, singly or in combination. The results will help modulate future treatment options if diagnosed with JIA and latent tuberculosis.

In the developing world, TB arthritis is an important cause of infectious arthritis and should be considered in a differential diagnosis of a child with arthritis. Skeletal involvement is seen in 1-3% of patients with half of these patients having spinal involvement and the rest extraspinal joint involvement. TB arthritis is usually monoarticular, usually involving larger, weight bearing joints and the organism can be isolated from the joint. Or it can present as a nondestructive polyarthritis that occurs during acute TB infection known as tubercular rheumatism or Poncet's disease in which no evidence of the organism can be found. [44]

HIV has been associated with a higher incidence of autoantibodies although not related to the occurrence of rheumatic manifestations. [42] HIV-associated arthritis can mimic JIA and must be considered if a patient is HIV positive. [42] Also of note is that anti-CCP and RF titer are raised nonspecifically in advanced HIV infection before immune reconstitution and thus are not useful in this scenario. [37]

Malnutrition results in impairment of immune system function. It influences both the innate as well as the acquired immunity. [2,45] Obesity shares with most chronic diseases the presence of an inflammatory component. [3] Both of these states of malnutrition will influence the immune system and influence our laboratory marker profile.

Systemic JIA:

Systemic JIA is both clinically and immunologically distinct from the other subgroups. In addition to arthritis, patients with sJIA have prominent symptoms of systemic inflammation such as fever, lymphadenopathy, organomegaly and serositis. As it is a diagnosis of exclusion and requires extensive and sometimes invasive testing there are normally delays in diagnosis and initiation of treatment. This can place the patient at risk for complications like macrophage activation syndrome (MAS).

Laboratory markers like ferritin, clotting profile and liver function tests can be used to determine whether MAS is present. Understanding of the pathogenesis of systemic JIA has been pivotal in identifying new immune markers to help speed up the process of diagnosis. [1]

The myeloid related proteins, MRP8 and MRP14, are calcium-binding proteins expressed in granulocytes, monocytes and macrophages during early differentiation stages, and can activate monocytes by binding to Toll-like receptor 4. TLR4 signaling will subsequently result in NFkB activation and TNF-alpha production, which contributes to inflammation. MRP8/14 protein complex, also known as calprotectin, has been shown to be useful in diagnosing systemic JIA in the presence of fever of unknown origin. It has been shown to be 44 times higher than in healthy controls. [1] Calprotectin concentrations distinguish systemic JIA from infection, with a specificity of 95%. Calprotectin serum concentrations can also be used to detect subclinical inflammatory activity and predicts relapse of the disease after therapy withdrawal. [1]

Conclusion:

According to the disease distribution within our study population, the most frequent subgroup of JIA diagnosed was poly RF- JIA (23.6%), closely followed by OligoJIA (23%). Patients with sJIA were shown to have baseline immune dysfunction with significant inflammatory profile with abnormal Hb, WCC and plt (although not statistically significant) and statistically significant elevated CRP levels compared to the other subgroups (P value 0.025). Patients with OligoJIA and sJIA presented earliest at a median age of 5 years and 5.5 years respectively. The laboratory marker profile of our patients is suggestive of a high infection burden.

ANA positivity was present in all subgroups, in varying percentages, except for the undifferentiated group where all patients tested had negative ANA. 9% of patients were underweight, 9% were overweight and 4.8% were obese. As nutrition is a critical determinant of immune responses, with both micro and macronutrient deficiency altering immunocompetence and increasing risk for infection, together with the knowledge that malnutrition is the most common cause of immunodeficiency worldwide, we need to consider the impact of malnutrition on the laboratory marker profile of patients due to immune modulation of nutrient deficiency. Conversely it is appreciated that overnutrition and obesity also contribute to an altered immunity. [2,3] Further studies are needed to elucidate the impact of immune activation and the resultant effect on the local reference ranges of baseline autoantibodies, namely rheumatoid factor (RF) and anti-nuclear antibodies (ANA).

Nonspecific ASOT elevation was seen in 12.7% of patients, most commonly in the systemic JIA subgroup, followed by poly RF- subgroup. The raised ASOT marker profile of our patients is suggestive of an associated Streptococcal infection burden

and may also act as a trigger. Baseline normal ranges of ASOT need to be established so that we may interpret the results in the context of our population where positive ranges in otherwise healthy patients may be higher. Further studies are needed to elucidate the impact of this high infection burden as a possible trigger for JIA and the resultant effect on immune modulation on the reference ranges of baseline autoantibodies, namely RF, ANA and ASOT so that we may interpret the results in the context of our population where normal ranges for the healthy patients may be higher.

The diagnosis of JIA remains predominantly a clinical one and the clinician needs to be cognisant of this when requesting laboratory markers as an aid to diagnosis. The clinical usefulness of laboratory markers especially in this context is driven in large by clinicians requesting behavior, the suspected subgroup of JIA, knowledge of the limitations of a given test, which includes sensitivity and specificity and the ability to interpret test results in the local clinical context. And the financial implications and appropriate use of resources need to be considered when requesting laboratory markers.

Suggested laboratory marker uses in patients with suspected JIA:

In patients presenting with oligoarthritis, i.e. less than or 4 involved joints, an ANA should be requested and if this is positive, titer >1:640 it is more indicative of oligoJIA and where there is a concern about possible SLE, where ANA is positive an anti-dsDNA test must be requested which is more specific.

In patients presenting with polyarthritis, i.e. 5 or more involved joints, a RF should be done to classify these patients as RF+ or RF- as RF is not only diagnostic but prognostic and reflects a group of JIA patients with a marked clinical picture and worse outcome if positive which is important for the patient and parents to know. Based on our study population RF negativity was a good predictor of anti-CCP negativity. Although you would want to test these patients for anti-CCP as it is indicative of an emerging adult phenotype where the patient is often sicker with more severe disease and a poorer outcome. Anti-CCP may be positive before RF is.

In patients presenting with suspected sJIA, laboratory markers such as Hb, WCC and plt as well as inflammatory markers, CRP and ESR, are useful baseline markers as they tend to be significantly raised and other markers such as serum ferritin are indicated early on as these patients are at a higher risk for macrophage activation syndrome.

HLA-B27 antigen should only be requested if ERA JIA is suspected.

For all patients it is advisable to do WCC, Hb, Plt and ALT as a baseline, to differentiate possible systemic JIA and as a baseline tests before starting treatment.

Baseline investigations for JIA should always include those for TB, HIV and post-infectious arthritis not only as diagnoses of exclusion but with potential effects on autoantibodies and implications for when immunosuppressive therapy is started.

Study Strengths:

This was a novel study focused on the description of baseline laboratory markers in patients with various subtypes of JIA in a population with a variety of ethnicities. It has provided new knowledge and insights into JIA, the demographics of our patients at presentation, the distribution of the 7 subgroups and range of routine baseline laboratory marker profile. The diagnosis of JIA, using the ILAR classification, was made by a paediatric rheumatologist and/or paediatric rheumatologist fellow. The use of the standard ILAR classification allows our study to be internationally comparable, safe and cost effective as it was a retrospective descriptive study.

Limitations:

Due to its retrospective nature some patient folders were not able to be retrieved and thus some of the data was incomplete. The study population was small. With regards to the laboratory markers, some of the markers came in to use many years after the opening of the clinic in 1995 and thus some patient's did not have all the above-mentioned markers measured, as well as the technique, instrumentation and cut-off value for the test kits changing for some of the laboratory markers over the years. We relied on the fact that the samples for the laboratory tests were taken and stored correctly. There was also a change in the laboratory information system used to store data, namely from DISA lab to NHLS LABTRACK with a resultant loss of some data. There is also the chance of bias as the suspected diagnostic category of JIA would have determined which laboratory tests were requested before referral to the clinic.

Polyarticular JIA, as the commonest subgroup in our cohort, was Rheumatoid factor negative predominant and systemic JIA demonstrated a significant inflammatory profile, with a significantly elevated CRP (P value 0.025) and elevated platelet counts, however not statistically significant. Nonspecific ASOT elevation was seen in 12.7% of patients, most commonly in the systemic JIA subgroup, followed by poly RF negative subgroup. ANA positivity was present in all subgroups, in varying percentages, except for the undifferentiated group where all patients tested had negative ANA. 9% of patients were underweight, 9% were overweight and 4.8% were obese. As nutrition is a critical determinant of immune responses, with both micro and macronutrient deficiency altering immunocompetence and increasing risk for infection, together with the knowledge that malnutrition is the most common cause of immunodeficiency worldwide, we need to consider the impact of malnutrition on the laboratory marker profile of patients due to immune modulation of nutrient deficiency. Conversely it is appreciated that overnutrition and obesity also contribute to an altered immunity. [2,3]

Raised ASOT marker profile of our patients is suggestive of an associated Streptococcal infection burden. Baseline normal ranges of ASOT need to be established so that we may interpret the results in the context of our population where positive ranges in otherwise healthy patients may be higher. Further studies are needed to elucidate the impact of immune activation and the resultant effect on the local reference ranges of baseline autoantibodies, namely rheumatoid factor (RF) and anti-nuclear antibodies (ANA). Baseline normal ranges of ASOT need to be established so that we may interpret the results in the context of our population where positive ranges in otherwise healthy patients may well be higher.

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Appendices:

Appendix1:

Haemoglobin g/dl			
Age	Gender	LoRange	HiRange
13Y	F	12.00	15.00
13Y	M	13.00	17.00
12Y	F	11.70	14.90
12Y	M	12.50	16.50
9Y		11.80	14.60
6Y		11.80	14.60
5Y		10.70	14.60
12M		10.70	13.10
6M		10.10	12.90
56D		9.10	13.10
29D		10.20	18.20
8D		14.90	22.90
1D		15.00	25.00

Appendix 2:

White Cell Count x 10^9/l			
Age	Gender	LoRange	HiRange
13Y		4.00	10.00
6Y		3.90	10.20
5Y		6.00	16.00
12M		6.00	18.00
6M		6.00	18.00
56D		5.50	18.00
29D		5.00	19.50
8D		5.00	20.00
1D		9.00	30.00
0D		9.00	30.00

Appendix 3:

Platelets x 10^9/l			
Age	Gender	LoRange	HiRange
13Y	male	178	400
13Y	female	137	373
12M		180	440
8D		140	350
1D		120	450
0D		120	450

Appendix 4:

ILAR Classification (2001):

General Definition of JIA:
Juvenile Idiopathic arthritis is arthritis of unknown etiology that begins before the 16th birthday and persists for at least 6 weeks, other known conditions excluded
Exclusions:
A: Psoriasis or a family history of psoriasis in the patient or a first degree relative
B: Arthritis in an HLA-B27 positive male beginning after the 6th birthday
C: Ankylosing spondylitis, enthesitis-related arthritis, sacroiliitis with inflammatory bowel disease, Reiter's syndrome, acute anterior uveitis or a history of one of these disorders in a first degree relative
D: The presence of IgM rheumatoid factor on at least two occasions three months apart.
E: Presence of systemic JIA in the patient.
Categories:

Systemic JIA:
Definition: Arthritis in one or more joints with or preceded by fever for at least two weeks duration that is documented to be daily (quotidian) for at least 3 days and accompanied by one or more of the following:

- Evanescent, non-fixed rash erythematous rash
- Generalized lymph node involvement
- Hepatomegaly and/or splenomegaly
- Serositis

Exclusions: a, b, c and d

Oligoarthritis:

Definition*:* Arthritis affecting one to 4 joints during the first 6 months of disease. Two subcategories are recognized:

1. Persistent oligoarthritis: Affecting not more than 4 joints throughout the disease course
2. Extended oligoarthritis: Affecting a total of more than 4 joints after the first 6 months of disease

Exclusions*:* a, b, c, d, e.

Polyarthritis (Rheumatoid Factor Negative):

Definition*:* Arthritis affecting 5 or more joints during the first 6 months of disease; a test for RF is negative.
Exclusions: a, b, c, d, e.

Polyarthritis (Rheumatoid Factor Positive):

Definition*:* Arthritis affecting 5 or more joints during the first 6 months of disease; 2 or more tests for RF at least 3 months apart during the first 6 months of disease are positive.
Exclusions: a, b, c, e.

Psoriatic Arthritis:

Definition*:* Arthritis and psoriasis, or arthritis and at least 2 of the following:

1. Dactylitis
2. Nail pitting or onycholysis
3. Psoriasis in a first-degree relative

Exclusions*:* b, c, d, e.

Enthesitis Related Arthritis:

Definition*:* Arthritis and enthesitis, or arthritis or enthesitis with at least 2 of the following:

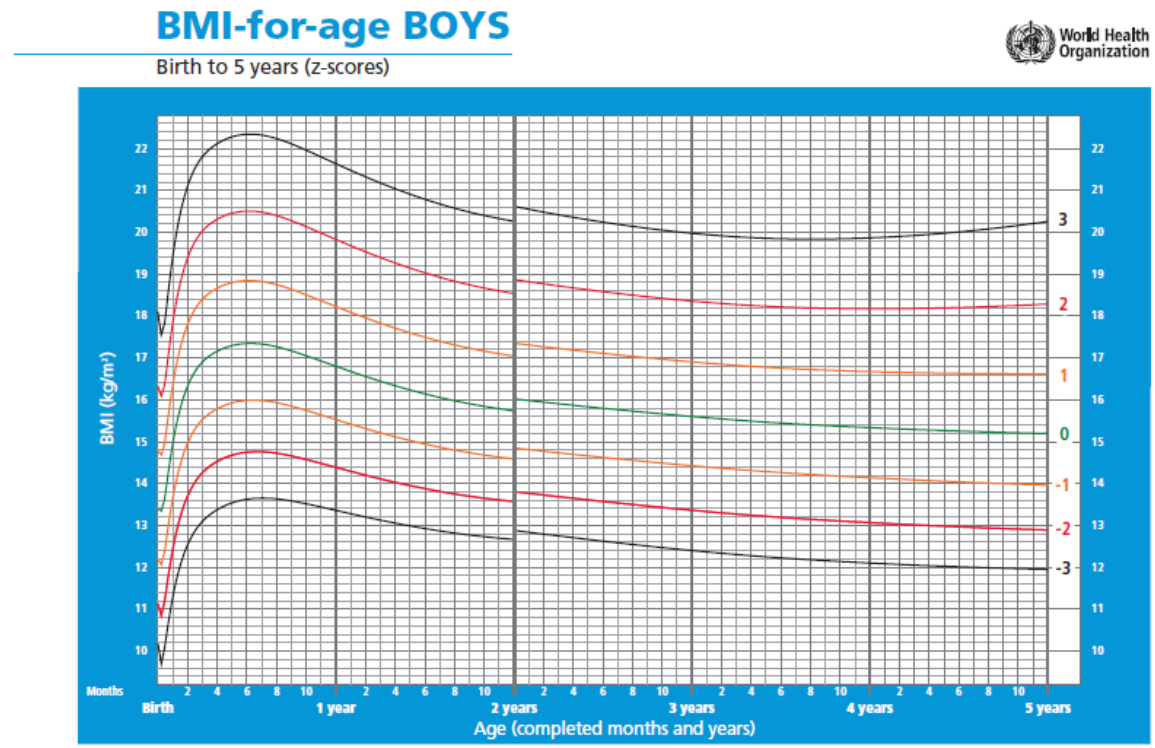
1. The presence of or a history of sacroiliac joint tenderness and/or inflammatory lumbosacral pain
2. The presence of HLA-B27 antigen
3. Onset of arthritis in a male over 6 years of age
4. Acute (symptomatic) anterior uveitis
5. History of ankylosing spondylitis, enthesitis related arthritis, sacroiliitis with inflammatory bowel disease, Reiter's syndrome, or acute anterior uveitis in a first-degree relative

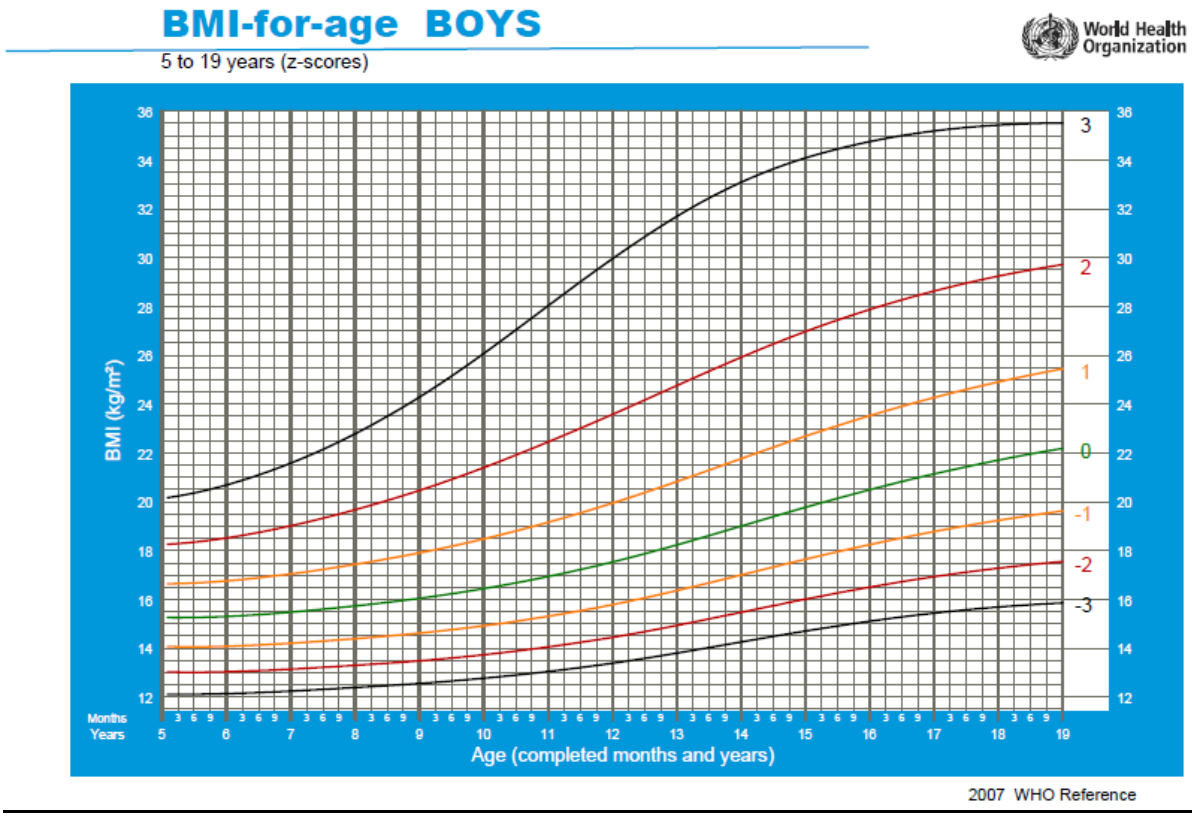
Exclusions*:* a, d, e.

Undifferentiated Arthritis:

Definition: Arthritis that fulfills criteria in no category or in 2 or more of the above categories.

Appendix 5: BMI charts:

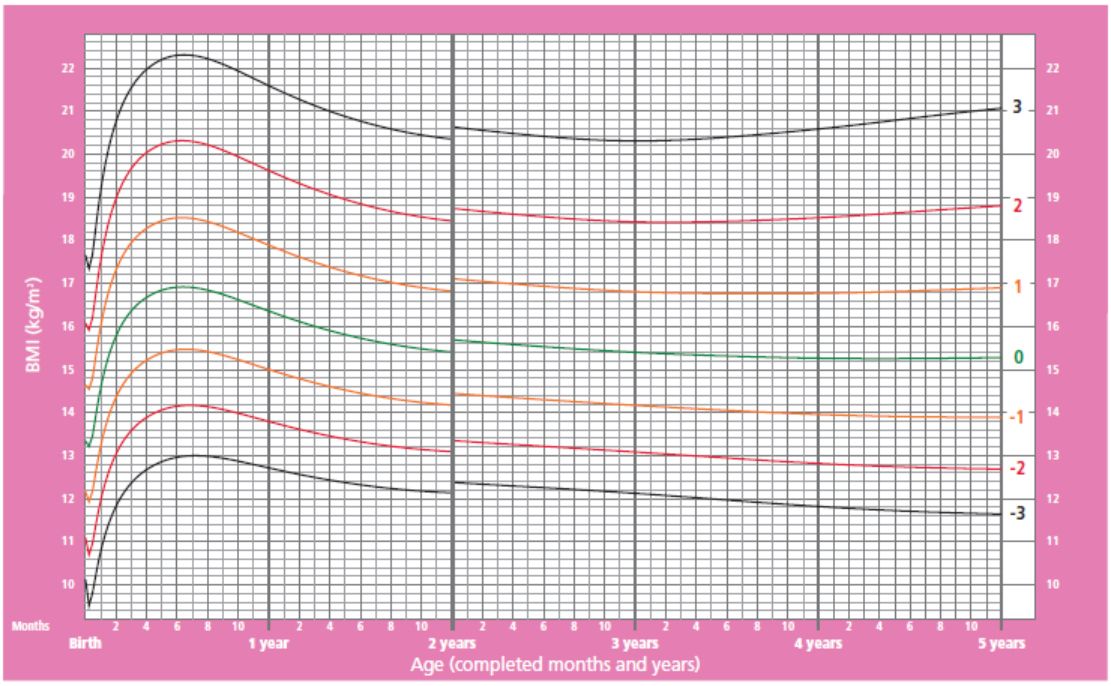




BMI-for-age GIRLS

Birth to 5 years (z-scores)

World Health Organization

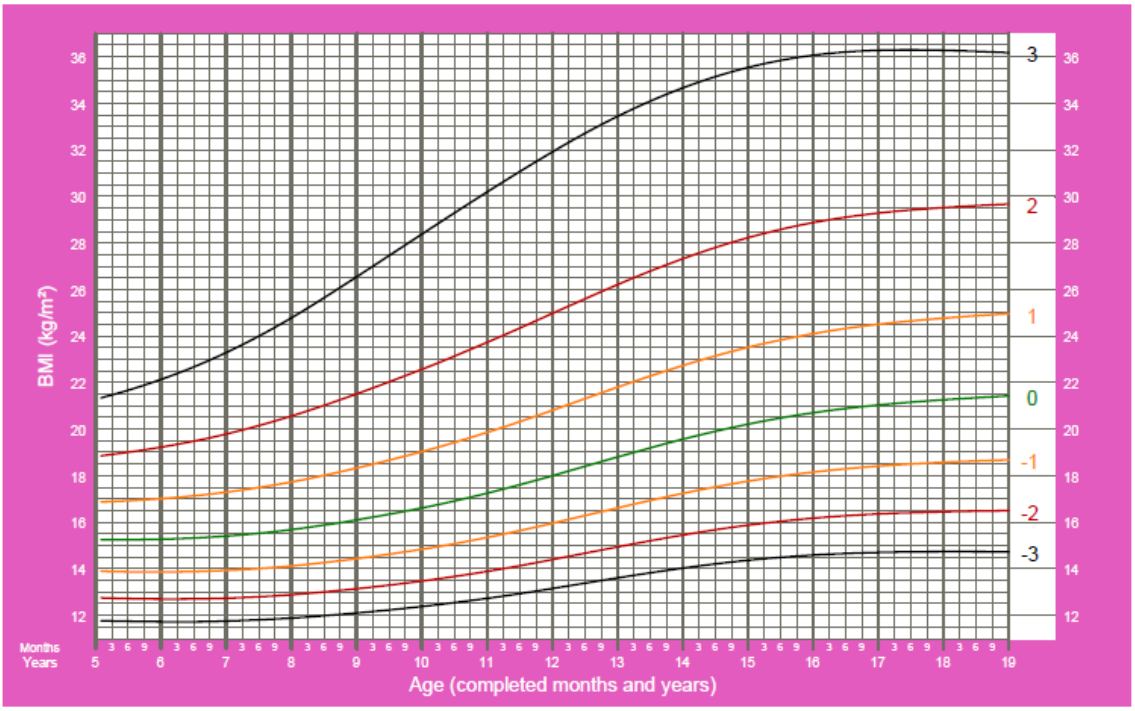


WHO Child Growth Standards

BMI-for-age GIRLS

5 to 19 years (z-scores)

World Health Organization



2007 WHO Reference

Tables:

Table 1:

Number of tests done across JIA sub-categories							
	OligoJIA [N=38]	PolyRF+ [N=26]	PolyRF- [N=39]	Systemic [N=14]	ERA [N=26]	Psoriatic [N=4]	Undiffer [N=18]
Full blood count	30	21	35	9	22	3	14
Full blood count and ALT	25	19	31	5	21	2	11
ASOT and anti-DnaseB	17	7	19	4	11	0	5
ESR and CRP	8	5	7	6	7	0	3
RF and ANA	32	26	38	12	22	2	15
HLA-B27 antigen	11	16	17	5	24	3	10

Table 2:

Laboratory marker profile across JIA sub-categories							
	OligoJIA [N=38]	PolyRF+ [N=26]	PolyRF- [N=39]	Systemic [N=14]	ERA [N=26]	Psoriatic [N=4]	Undiffer [N=18]
RF [number of seropositive/tested] (%)	1/32 (3%)	26/26 (100%)	3/39 (7.7%)	0/12	3/23 (13%)	0/3	2/15 (13.35%)
ANA [number of seropositive/tested] (%)	11/35 (31.4%)	6/26 (23%)	6/38 (7.9%)	1/12 (8%)	1/22 (5%)	2/2 (100%)	0/16
ASOT [number of seropositive/tested] (%)	4/19 (21%)	1/7 (14.2%)	11/22 (50%)	3/5 (60%)	1/11 (9%)	0/1	1/7 (14%)
HLA-B27 [number of seropositive/tested] (%)	0/11	1/16 (6%)	3/17 (18%)	0/5	14/24 (58%)	0/3	4/10 (40%)
Anti-DNAsB [number of seropositive/tested] (%)	11/18 (61%)	2/7 (29%)	14/19 (74%)	2/4 (50%)	6/11 (55%)		3/5 (60%)
DsDNA [number of seropositive/tested] (%)	0/8	2/8 (25%)	1/8 (13%)	0/3	0/2		0/4
Anti-CCP [number of seropositive/tested] (%)	0/12	14/22 (64%)	0/11	1/5 (20%)	0/12	0/1	0/5
CRP (ug/dl) median	6/13 (46%) 57	3/5 (6%) 14	4/9 (44%) 58	7/7 (100%) **150**	2/7 (29%) 60	0	1/3 (33%) 11
ESR (mm/hr) median	19/28 (68%) 28	18/23 (78%) 40	27/35 (77%) 49	7/10 (70%) 79	16/22 (73%) 50	2/4 (50%) 49	7/14 (50%) 25.0
Haemoglobin (g/dl)	12	12	12	9	12	13	13
White cell count (10^9/l)	10	9	8	18	9	8	7.0
Platelet (10^9/l)	383	374	427	638	405	279	331.0

Table 3:

Cost of laboratory markers (average cost)							
	OligoJIA	**PolyRF+**	**PolyRF-**	**Systemic**	**ERA**	**Psoriatic**	**Undiff**
Full blood count	R1740	R630	R2262	R522	R1276	R174	R812
Full blood count and ALT	R2575	R1957	R3193	R515	R2163	R206	R1133
ASOT and anti-DnaseB	R1768	R728	R1976	R416	R1144	R0	R520
ESR and CRP	R800	R500	R700	R600	R700	R0	R300
RF and ANA	R6784	R5512	R8056	R2544	R4664	R424	R3180
HLA-B 27 antigen	R770	R1120	R1190	R350	R1680	R210	R700

Abbreviations:

ALT: alanine aminotransferase
ANA: antinuclear antibody
Anti-CCP: anti-cyclic citrullinated peptide antibody
Anti-DNaseBB: anti-deoxyribonuclease B antibody
ASOT: antistreptolysin O titer
BMI: body mass index
C3: complement 3
C4: complement 4
CRP: C-reactive protein
DISA LIS: DISA laboratory information service
Ds-DNA: double stranded DNA
EBV: Epstein-Barr Virus
ENA: extractable nuclear antigen
ERA: enthesitis related JIA
ESR: erythrocyte sedimentation rate
FBC: full blood count
FN: false negative
FP: false positive
Hb: haemoglobin
HIV: human immunodeficiency virus
HLA-B27: Human leukocyte antigen B27
ILAR: International League of Associations for Rheumatology
JIA: Juvenile Idiopathic Arthritis
NGT: nominal group technique
NHLS: National Health Laboratory Service
OligoJIA: Oligoarthritis JIA
Parvo: Parvo virus
Plt: platelet count
Poly RF-: polyarticular rheumatoid factor negative JIA
Poly RF+: polyarticular rheumatoid factor positive JIA
PRINTO: Pediatric Rheumatology International Trials Organization
PsJIA: psoriatic JIA
RF: Rheumatoid factor
sJIA: systemic JIA
TB: tuberculosis
TN: true negative
TP: true positive
Undiff: undifferentiated JIA
WCC: white cell count