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Overview of care in cleft lip and palate for orthodontic treatment

Inês Neves Francisco

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Overview of care in cleft lip and palate for orthodontic treatment

Thesis presented at the Faculty of Dental Medicine of University of Porto for Doctor of Dentistry Degree

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Preface

In the earliest documented history, it was believed that many craniofacial malformations, including the cleft lip and palate, would be a consequence of an evil spirit in the affected child. Even today, some African tribes leave children with facial malformations to die in the surrounding wilderness.

The clinical features of cleft lip and palate (CLP) patients have several consequences in hearing, feeding, speech, dentofacial development, appearance, and adverse outcomes for social integration. Furthermore, CLP patients have higher morbidity and mortality throughout life than healthy individuals. The multiplicity of these clinical features requires a multidisciplinary care from birth until adulthood, which includes several medical specialties, namely plastic surgery, maxillofacial surgery, otolaryngology, speech therapy, psychology, genetics, orthodontics and dentistry.

The rehabilitation of CLP patients has a cost in terms of morbility, emotional and social disturbance, health care and employment exclusion, which considerable affect CLP patients, their families and society. Research in etiology, treatment and prevention can help to reduce the costs and adapt the available resources (personnel, time, facilities and equipment).

Several studies have been done to understand the etiology of CLP, but many predisposing factors are still unknown or controversial. Further studies are necessary in mothers of patients with and without CLP, in order to compare which maternal factors lead to a greater probability of having a cleft child. Knowledge of etiologic factors will help clinicians to predict the occurrence of the cleft and develop prevention measures.

Additionally, standards of care for CLP patients remain a cause for concern. Thus, it is important to analyse and compare the current treatment of CLP patients in Europe, identifying knowledge gaps and clinical needs in orthodontic care.

The CLP treatment requires a prolonged orthodontic treatment to achieve a good aesthetic result. Based on literature, CLP patients tend to have a lower oral health-related quality of life (OHRQoL) than a non-cleft population. However, there is no conclusion in the literature regarding the potential effect of orthodontic treatment on OHRQoL in CLP patients.

Moreover, tissue regeneration is a new emerging approach in orthodontics because a high percentage of patients need both regeneration and orthodontic treatment. Bone and soft tissue regeneration may be indicated for managing defects subsequent from several conditions, such as congenital defects (cleft lip and palate). Additionally, orthodontic tooth movement results from application of mechanical force on the teeth, which affects the periodontal ligament and the alveolar bone. A defect in support structures, as occurs in cleft lip and palate patients, may interfere with orthodontic tooth movement. Some regenerative therapies have been subject of study in CLP defects, namely platelet-rich fibrin (PRF). The use of PRF can improve orthodontic treatment results, as it releases growth factors over time, promoting a biological response with a minimally invasive procedure. Although, no conclusion of the potential effect of PRF on this process could be drawn based on limited literature.

This thesis aims to identify knowledge gaps on etiology, surgical and orthodontic treatment in patients with CLP based on the high clinical evidence. The results are expected to be beneficial for the CLP patients who can benefit from a widely accepted and effective treatment in the future.

Resumo

Introdução: As fendas orofaciais são as malformações congénitas da cabeça e do pescoço mais comuns, com uma prevalência mundial estimada de 14/10 000 nados-vivos. A fenda lábio palatina (FLP) apresenta uma multiplicidade de características clínicas com notórias implicações a nível da estética facial, audição, alimentação, fala, desenvolvimento dentofacial e psicossocial. O tratamento desta patologia é inevitavelmente multidisciplinar acarretando prejuízos económicos, na qualidade de vida e na autoestima destes doentes, com impacto direto no indivíduo, no seu núcleo familiar e na sociedade. Deste modo, a investigação científica e clínica direcionada para a etiologia, tratamento ortodôntico e novas técnicas de regeneração de tecidos, para além de aumentar o bem-estar e saúde destes doentes poderá contribuir para a redução dos custos individuais, familiares e sociais desta patologia através da melhor gestão dos recursos disponíveis.

Objetivos: (1) Avaliar a influência dos fatores de risco parentais no aparecimento da FLP bem como as diferenças dos dados antropométricos à nascença; (2) Avaliar o estado atual do atendimento de doentes com FLP no contexto Europeu; (3) Avaliar a qualidade de vida dos doentes com FLP, submetidos a tratamento ortodôntico, e respetivo núcleo familiar; (4) Avaliar os efeitos das novas técnicas de regeneração tecidular em doentes com FLP.

Métodos: Para o primeiro objetivo foi realizada a caracterização dos fatores de risco e dados à nascença através de um estudo caso-controlo que incluiu 266 doentes com FLP. Para a concretização do segundo objetivo foi realizado um questionário europeu para avaliar diversos aspetos entre os quais: características do prestador privado ou público, perfil do doente, perfil da equipa multidisciplinar e protocolo de tratamento. A avaliação da qualidade de vida dos doentes portadores de FLP, submetidos a tratamento ortodôntico, e dos seus familiares foi realizada através de dois questionários (Perfil de Impacto da Saúde Oral e a Escala de Impacto Familiar). Por fim, para realizar o quarto objetivo foi efetuada a avaliação dos efeitos das novas técnicas de regeneração através de duas revisões sistemáticas e de um estudo ex-vivo, em fémures de embriões de pinto.

Resultados: (1) Verificou-se que o aumento de cada ano da idade materna diminuiu a probabilidade de ter um filho com FLP em 0.9 (OR=0.903), mas o aumento de índice de massa corporal aumenta a probabilidade em 1.14 (OR=1.14). Relativamente aos dados antropométricos à nascença, verificou-se que por cada quilograma a mais ao nascimento, a probabilidade de ter um filho com FLP diminui 0,4 (OR=0.435). (2) Apesar das melhorias significativas que se verificaram na Europa, são ainda identificáveis algumas discrepâncias, nomeadamente na cronologia do tratamento e na aparatologia oferecida. Dos 69 inquiridos foram reportados 50 protocolos de tratamento. O sistema de referenciação mais utilizado é de centralização de serviços e, a maioria dos países incluídos refere possuir uma associação de FLP para os doentes e/ou para profissionais (53.6%). O Serviço Nacional de Saúde apoia a maioria dos cuidados ortodônticos em FLP (67%) na Europa. (3) A qualidade de vida durante o tratamento ortodôntico não é afetada pela condição de ser portador de FLP. No entanto, os pais de filhos com esta condição reportam pior qualidade de vida do que os seus filhos ou pais de crianças sem FLP. (4) A proteína morfogenética do osso e o enxerto ósseo autógeno apresenta uma eficácia semelhante em relação ao volume, preenchimento e altura do osso no enxerto secundário em indivíduos com FLP. A fibrina rica em plaquetas pode melhorar a reconstrução da fenda alveolar e diminuir o tempo de tratamento ortodôntico, reduzindo os custos associados.

Conclusão: A promoção da prevenção e a implementação de tratamentos com eficácia demonstrada, são dois eixos fundamentais para melhorar a qualidade de prestação de cuidados de saúde e o custo-benefício dos tratamentos. Nas duas últimas décadas verificou-se uma melhoria significativa nos cuidados de saúde cirúrgicos e ortodônticos em toda a Europa, mas é ainda necessário que estes tratamentos sejam suportados com um maior grau de evidência científica.

ABSTRACT

Introduction: Orofacial clefts are one of the most common craniofacial malformations, with an international prevalence in newborns of 14 per 10 000 live births worldwide. The clinical features of cleft lip and palate patients have several consequences in facial aesthetics, hearing, feeding, speech, dentofacial and psychosocial development. The rehabilitation of cleft lip and palate (CLP) patients is inevitably multidisciplinary, having a cost in terms of economics, quality of life and self-esteem of these patients, with a direct impact on CLP patients, their families and society. Scientific and clinical research in etiology, orthodontic treatment and new emerging tissue regeneration will promote an increase in the well-being and health of these patients, which will contribute to reducing costs in individuals, family and society through better adaption of available resources.

Objectives: (1) Evaluate the effect of possible parental related influencing factors on the development of cleft lip and/or palate as well as the differences in birth data; (2) Instigate a critical appraisal of cleft care in Europe; (3) Evaluate the levels of oral health-related quality of life (OHRQoL) in CLP patients undergoing orthodontic treatment and their families; (4) Evaluate the clinical effectiveness of regenerative strategies on the treatment of CLP patients.

Methods: For the first objective, the characterization of risk factors and child birth data of a population of 266 individuals with CLP was carried out through a case-control study. To achieve the second objective, a European survey was created with a unique URL to investigate several aspects of cleft care, i.e. provider characteristics, patient profile, multidisciplinary team profile and treatment protocol. To the assessment of the quality of life of CLP patients, 226 individuals (111 with cleft and 115 control) and their parents were invited to complete the Oral Health Impact Profile-14 and Family Impact Scale, respectively. For the fourth objective, the effectiveness of the regenerative strategies was carried out through two systematic reviews and in embryonic femurs cultured ex vivo.

Results: (1) Regarding parental related factors with statistical significance, it was verified that: for each maternal year increase, the probability of having a cleft child decreases 0.9 (OR=0.903); for each body mass index unit (kg/m^2) increase, the probability of having a cleft child increases 1.14 (OR=1.14). On the child data birth, it was showed that for each mass unit (kg) increase at birth, the probability of having a cleft child decreases 0.4 (OR=0.435). (2) A significant improvement in surgical and orthodontic healthcare has undergone all over the Europe but there are several discrepancies, namely regarding the treatment timing protocol and the appliances offered. Of the 69 answers, this survey showed 50 different treatment protocols. The centralization system was the preferred system and the majority of the countries have a cleft association for patients and professionals (53.6%). National Health Service supports the majority of cleft orthodontic care (67%) in Europe. (3) Undergoing orthodontic treatment had a similar impact on the overall quality of life in CLP patients and non-cleft patients. Parents of cleft children had a poorer OHRQoL compared to what was perceived by their children and parents of non-cleft children. (4) The effectiveness of tissue regeneration approaches was also investigated, with human bone morphogenetic protein and autogenous bone graft have similar effectiveness regarding volume, height and bone filling in oral cleft patients' surgery. Platelet rich fibrin can improve alveolar cleft reconstruction and may shorten the orthodontic treatment time reducing associated costs.

Conclusion: Two strategies can be used to reduce costs and improve the quality of healthcare in cleft care: prevention and the implementation of treatments with demonstrated efficacy. In the past two decades, a significant improvement in surgical and orthodontic healthcare has undergone all over the Europe but scientific evidence in cleft treatment is still need to be improved.

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Abbreviations

ACPA	American Cleft Palate Association
ADM	Acellular dermal matrix
AMSTAR 2	Assessment of Multiple Systematic Reviews
BMD	Bone mineral density
BMI	Body mass index
BMMNCs	Autologous bone marrow mononuclear cells
BMP-2	Bone morphogenic protein 2
BS	Bone surface
BV	Bone volume
CBCT	Cone beam computed tomography
CCTs	Controlled clinical trials
CI	Confidence interval
CL	Cleft lip
CL/P	Cleft lip with or without cleft palate
CLP	Cleft lip and palate
CP	Cleft palate
CR	Case report
CS	Case series
CT	Clinical trial
CT	Computed tomography
DBB	Deproteinized bovine bone
DBM	Demineralized bone matrix
DDM	Demineralized dentinal matrix
EUROCAT	European Network for Epidemiological Surveillance of Congenital Anomalies
F	Female
FIS	Family Impact Scale
FLP	Fenda lábio-palatina
FMUC	Faculty of Medicine, University of Coimbra
HA	Hydroxyapatite
HROB	High risk of bias
ICBG	Iliac cancellous bone graft
IRF6	Interferon regulatory factor 6
L-PRF	Leucocyte and platelet-rich fibrin
LROB	Low risk of bias
M	Male
MA	Meta-analysis

MD	Mean difference
MSC	Mesenchymal stem cell
N-RCT	Non-randomized controlled trial
NAM	Nasoalveolar molding
NOS	Newcastle-Ottawa scale
NSCs	Neural stem cells
OC	Orofacial clefts
OHIP-14	Oral Health Impact Profile-14
OHRQoL	Oral Health-Related Quality of Life
OMIM	Online Mendelian Inheritance in Man
OR	Odds ratio
OTM	Orthodontic tooth movement
p	p-value
PAOO	Periodontally accelerated osteogenic orthodontics
PAX7	Paired box protein Pax-7
PDGF	Platelet-derived growth factor
PICO	Population, Intervention, Comparison and Outcome
PRF	Platelet-rich fibrin
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-analyses
PRP	Platelet-rich plasma
PS	Prospective study
PSO	Presurgical maxillary orthopaedics
RCR	Retrospective controlled review
RCT	Randomised controlled trial
rhBMP-2	Recombinant human bone morphogenetic protein–2
ROB	Risk of bias
RS	Retrospective study
SD	Standard deviation
SEM	Scanning electron microscopy
SMD	Standardized mean difference
SR	Systematic review
TGF-β1	Transforming growth factor-beta1
USA	United States of America
VAX1	Ventral anterior homeobox 1
VEGF	Vascular endothelial growth factor
β-TCP	β-tricalcium phosphate
μCT	Microtomographic

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Chapter 1

Introduction

1. Prevalence

Orofacial clefts (OC) are one of the most common craniofacial malformations, with an international prevalence in newborns of 14 per 10 000 live births worldwide.[1-2] Prevalence can be affected by sex, racial and ethnic groups, geographical regions, and socioeconomic conditions.[2-5]

Previous studies reported that the prevalence of cleft lip and palate (CLP) is higher in Asians and Native Americans and lower in Africans and Southern Europeans.[3,5] Moreover, the prevalence in cleft palate (CP) appears to be similar in Europeans, Africans, Native Americans and Asians.[2,6] The reported prevalence per 10000 births was: East Asians: 13.1 to 31.8; Native Americans: 5.5 to 25; Africans: 3.2 to 4.6; Caucasians: 6.9 to 23.5; and, African Americans: 1.8 to 8.2.[3]

The prevalence of OC has been increasing over the years, which might be related with several factors, among them, the improved surgical techniques and neonatal care resulting in reduced postnatal morbidity and mortality, more accurate documentation and environmental factors such as maternal corticosteroids use or smoking and alcohol consumption.[7-9]

According to the European Network for Epidemiological Surveillance of Congenital Anomalies (EUROCAT) report, the prevalence in 26 European countries was 14.5 per 10 000 births between 2011 and 2018 (Figure 1.1).[10]

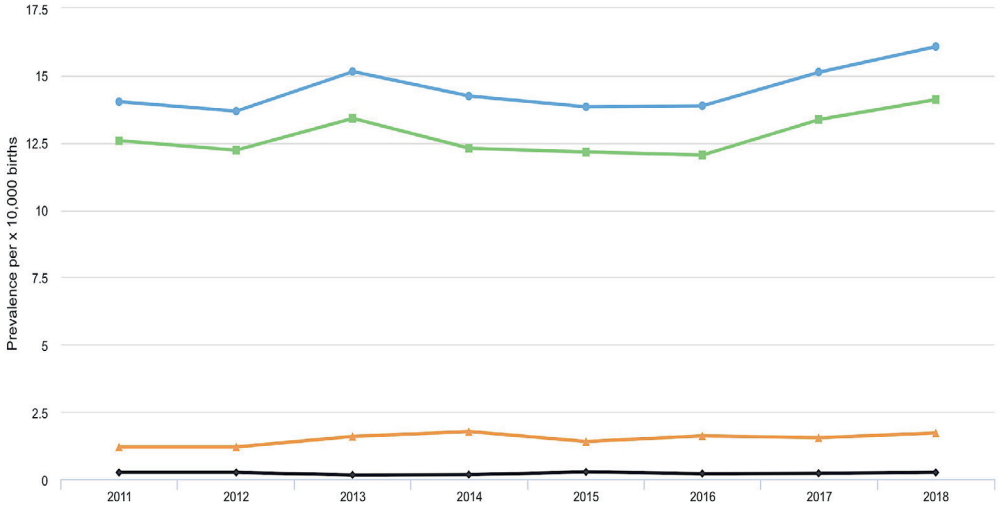

Figure 1.1. Prevalence per 10 000 according EUROCAT: blue line- all cases; green line- live births; orange line- termination of pregnancy for congenital anomaly; black line- fetal deaths / still births from 20 weeks gestation.[10]

The data derived from the 2015 Portuguese National Registry of Congenital Birth Defect registered this trend, since the prevalence increased from 6.2 to 7.7 per 10 000 live births in the 2011-2013 and 2014-2015 report, respectively.[11]

The prevalence among phenotypes is variable, being higher in CLP than cleft lip (CL) or CP. Regarding sex, CLP is twice as frequent in males than in females, while CP is 1.5 times more common in females.[12-13] About 52% of them are left-sided, 24% right-sided and 24% are bilateral.[3]

2. Developmental pathogenesis

An understanding of the development of craniofacial region is crucial to comprehend the changes resulting from this malformation. The development of the face occurs in an early embryonic stage and entails a complex series of events that require cell migration, growth, differentiation, and apoptosis in an extremely coordination manner.[13] In the four-week of intrauterine life, the development of the human face begins with the migration of neural crest cells from the dorsal region of the anterior neural tube through mesenchymal tissue in facial region, developing the frontonasal processes, two maxillary processes, and two mandibular processes. The fronto-nasal prominence will form the nose, forehead, and the upper limit of the primitive mouth. The paired maxillary processes will constitute the lateral sides of the stomodeum and the paired mandibular processes will form the caudal boundaries.[14-15]

By the end of four-week, formation of the nasal placodes occurs by ectodermal thickenings, dividing the lower portion of the frontonasal processes into medial and lateral nasal prominences, which are the precursors of the nose. The lower portions of the medial nasal prominences are also involved in the development of the upper lip, alveolar ridge that surrounds the upper incisors and part of the hard palate (primary palate). Toward the end of the six-week of intrauterine life, maxillary process proliferates, which causes the nasal structures to move medially and, in this way, the medial nasal prominences and the maxillary process begin to merge area to form a vertical groove in the middle of the upper lip (lip philtrum). By the eight-week, both maxillary processes develop forward and fuse with the lower edges of the lateral nasal prominences, below the nasal pits to reach and fuse with the vertical groove of the upper lip, producing a continuous ridge (upper lip). During this process, the lateral nasal process shows a peak of cell division to reinforce the fused tissues. Although, if any growth disturbance occurs, causing a fusion failure, a unilateral or bilateral lip cleft will result.[14-16]

The development of the secondary palate begins at the fifth week with the outgrowth of two palatine shelves of the maxillary process that initially grow down on each side of the tongue. At the same time, the mandible grows downward and forward, leading the tongue to adopt a more

downward position. By the seventh week, the palatine shelves rise to a horizontal position above the tongue, which permits the fusion between the nasal septum and primary palate. Two weeks after, the palatal shelves start the fusion with the free edges of the nasal septum in an anterior-to-posterior sequence. By the twelfth week, the fusion is complete, forming the hard palate and most posterior region that do not ossify (the soft palate and the uvula).[14-16] Between the sixth and ninth weeks, a failure in the fusion of palatal shelves may happen, resulting in a cleft palate.[17] Figure 1.2 presents a schematic diagrams of human craniofacial development.

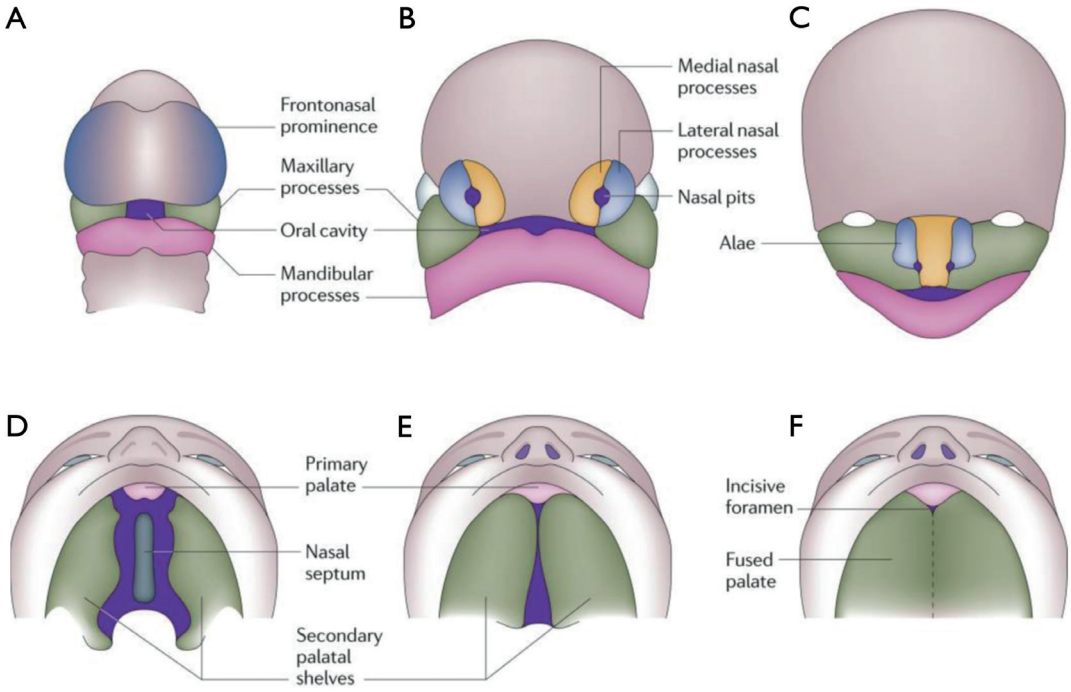

Figure 1.2. Schematic diagrams of human craniofacial development. (A) Fourth week- formation of the frontonasal processes, two maxillary processes, and two mandibular processes; (B) formation of the nasal placodes (medial and lateral nasal processes); (C) between the sixth and eighth week- formation of the upper lip and primary palate; (D) sixth week- growing of two palatine shelves; (E) seventh week- elevation of palatal shelves to a horizontal position; (F) fusion of the secondary palatal shelves.[17]

3. Etiology

The etiology of CLP is not completely known but several genetic and environmental risk factors are identified.[17]

Environmental risk factors

Previous researches have proposed smoking consumption during pregnancy and gestational diabetes as maternal risk factors and have been rather consistent.[8,18] Maternal smoking increased the risk of clefting in approximately 20% (odds-ratio of 1.3 for CLP).[8] Little at al. showed that the relationship between maternal tobacco smoking and OC is strong enough to support anti-smoking campaigns.[19] A recently largest population-based study showed that mothers with extremes in pre-pregnancy weight are more likely to have a newborn with cleft lip and/or palate.[18]

Other associations have been reported with less consistency, namely alcohol consumption during pregnancy[20-21], intrapartium interval[22], parity[23], maternal and paternal age[23-24], nutritional deficiencies (folic acid, vitamin A and B12 and zinc) and changes in the amniotic fluid[25-27]. Among the teratogenic agents most associated with CLP, exposure to ionizing radiation, carbon monoxide, polycyclic aromatic hydrocarbons and some drugs, namely valproic acid, retinoids, phenytoin, antiepileptic agents or coticosteroids have been suggested.[28-31] However, some of these risk factors do not have a consensus in the literature due to the lack of comprehensive investigation that may have contributed to ambiguous reports.

Genetic risk factors

Regarding CLP genetics, it is important to distinguish syndromic from nonsyndromic orofacial clefts. Patients are considered syndromic if they have other physical or cognitive abnormalities.[32] Accordingly to Online Mendelian Inheritance in Man (OMIM) at least 275 syndromes exist, in which clefting is a primary and these are caused by mutation of a chromosomal abnormalities, teratogens

or single genetic locus.[33] Van der Woude syndrome is the most common form of syndromic clefting and is caused by mutations in Interferon Regulatory Factor 6 (IRF6), presenting a prevalence of approximately 2% of all CLP cases.[34] Most orofacial cleft cases, approximately 75% of CLP and 50% of CP, do not have additional features and are categorized as nonsyndromic clefting syndromes and affect one in several hundred thousand live births.[33,35-36] Table 1.1 summarizes some clefting syndromes and their genetic locus.

Table 1.1. Cleft lip and/or palate syndrome. Adapted from Leslie et al.[33]

Syndrome	Cleft Type	Gene	Reference
Apert	CP	FGFR2	Wilkie et al.[37]
CHARGE	CP	CHD7	Vissers et al.[38]
CLP ectodermal dysplasia	CL/P	PVRL1	Suzuki et al.[39]
Crouzon	CP	FGFR2	Reardon et al.[40]
DiGeorge	CP	TBX1	Packham and Brook[41]
Ectrodactyly-ectodermal dysplasia-clefting	CL/P	TP63	Celli et al.[42]
Familial gastric cancer and CLP	CL/P	CDH1	Frebourg et al.[43]
Kabuki	CL/P	MLL2 KDM6A	Lederer et al.[44]
Oculofaciocardiodental	CP	BCOR	Ng et al.[45]
Pierre Robin	CP	SOX9	Benko et al.[46]
Tooth agenesis with or without cleft	CL/P	MSX1	Van den Boogaard et al.[47]
Treacher Collins	CP	TCOF1	Group 1996[48]
Van der Woude	CL/P	IRF6	Kondo et al.[49]
X-linked cleft palate and ankyloglossia	CP	TBX22	Braybrook et al.[50]

CL- cleft lip; CP- cleft palate; CL/P- cleft lip with or without cleft palate

The genetic component plays an important role in the formation of isolated cleft, but in contrast to syndromic clefting, this occurrence is due to complex and multifactorial inheritance. Several studies based on segregation analysis and twin studies demonstrated a genetic component in nonsyndromic cleft.[33, 51] Sivertsen et al. showed that the risk for CL in first-degree relatives is estimated to be 32 times higher than individuals without a family history of CL.[52] Furthermore, the concordance rate in dizygotic twins (3-5%) is lower than monozygotic twins 40–60%, which suggests a genetic etiology.[53] Several candidate genes have been discovered that contribute to nonsyndromic cleft, using a diversity of approaches, namely genome-wide association studies, linkage analysis, genomic rearrangements and candidate genes.[14,54]

Some of the best-supported genes and genetic loci will be mentioned following:

1) IRF6 was first implicated in two autosomal-dominant clefting syndromes. Although, some hypomorphic alleles in IRF6 have been suggested in the etiology of nonsyndromic cleft.[55-57]
2) ch8q24 region was associated with nonsyndromic cleft in Caucasian populations. The closest gene to this locus is the MYC, which is involved in neural crest cell formation and craniofacial development of mandible and maxilla.[58-60]
3) Ventral anterior homeobox 1 (VAX1) is a transcriptional regulator containing a DNA-binding. Vax1 was described to be associated with the development of cleft palate.[61]
4) Paired box protein Pax-7 (PAX7) is a transcription factor that regulates the expression of neural crest markers Slug, Sox9, and Sox10. PAX7 can affect the craniofacial development as it is expressed in nasal placodes, Meckel's cartilage and other nasal structures including the nasal epithelium.[62-63]

Gene-environmental risk factors

Recently, several studies aimed to estimate the main effects of genes-environment interaction. Some of them suggested an increase in the risk of cleft, such as: alcohol and ADH1C variants; MTHFR polymorphisms and maternal folate intake; NAT1 1095 polymorphism and lack of maternal multivitamin; maternal smoking and Transforming Growth Factor Alpha.[64-67] So far, the results of these interactions are inconclusive due to low statistical power analyse, study heterogeneity, absence of replication work, differences in genotyped samples (eg mother alone or with her child), differential assessment of environmental exposures and research limited to a few industrialised countries populations.[13]

4. Classification

Classification of the cleft is crucial to international communication of cleft lip and palate patients. Davis and Ritchie presented the first standard system of classification, using the alveolar process as the reference to typify the three-group (Group 1- Prealveolar process cleft; Group 2- Postalveolar process cleft; Group 3- Alveolar process cleft). Since that, several classifications have been proposed based on embryology or morphological characteristics.[68] In 1973, Spina proposed a classification having the incisive foramen as anatomical basis, in which He divided the clefts into[69]:

1. **Pre-incisive foramen cleft-** Clefts of lip and palate anterior to the incisive foramen:
   a. Unilateral - left or right (Figure 1.3), median or bilateral;
   b. Total or partial

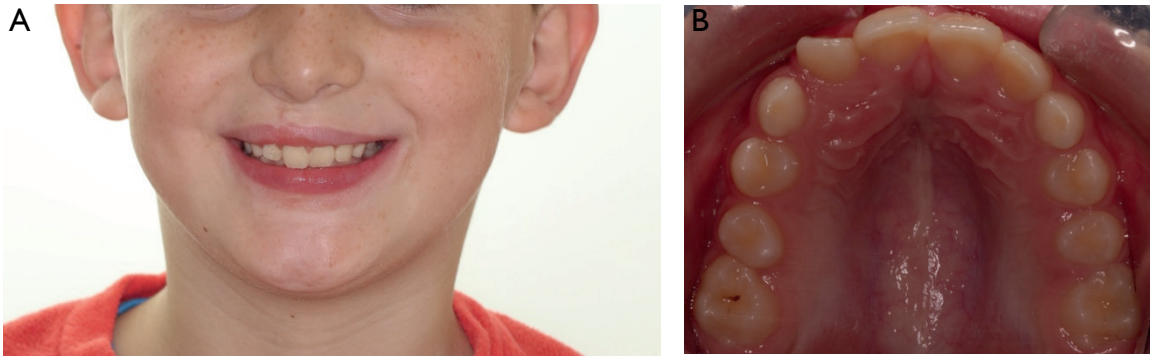

Figure 1.3. Cleft lip on the right side: extra-oral photography (A); intra-oral occlusal photography (B).

2. **Trans-incisive foramen clefts-** Clefts of the lip, alveolus and palate:
   a. Unilateral- left (Figure 1.4), right (Figure 1.5) or bilateral (Figure 1.6).

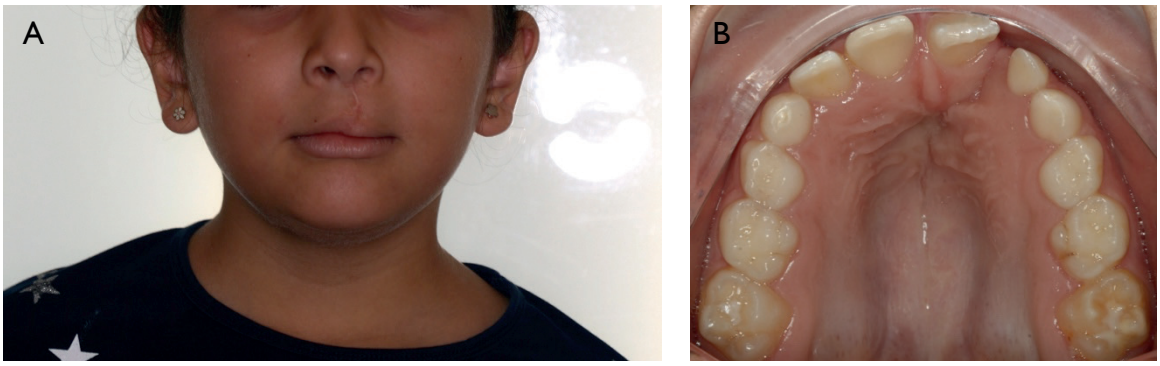

Figure 1.4. Unilateral Cleft lip and palate on the left side: extra-oral photography (A); intra-oral occlusal photography (B).

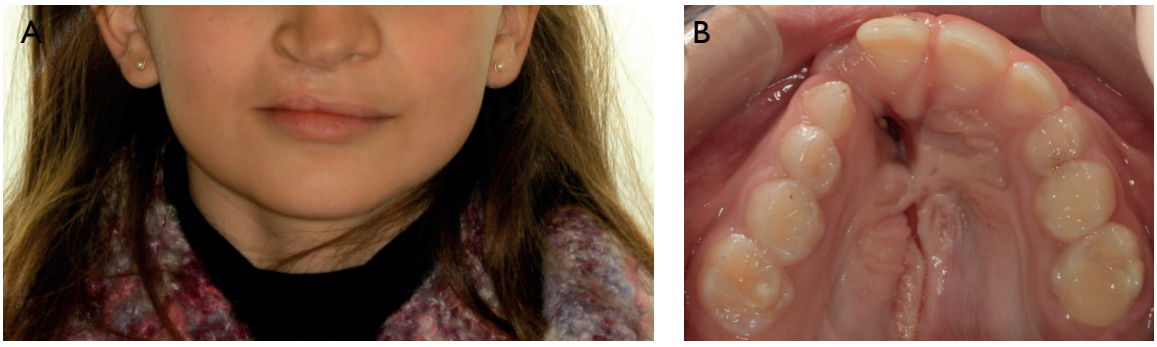

Figure 1.5. Unilateral Cleft lip and palate on the right side: extra-oral photography (A); intra-oral occlusal photography (B).

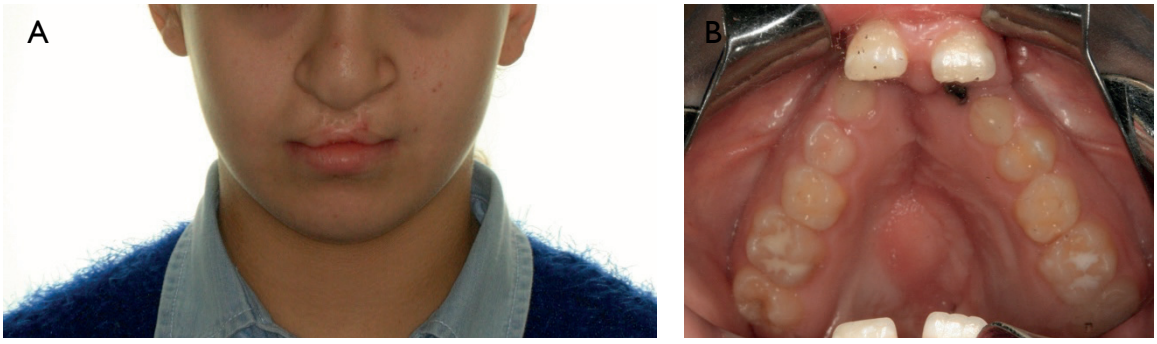

Figure 1.6. Bilateral Cleft lip and palate: extra-oral photography (A); intra-oral occlusal photography (B).

3. **Post-incisive foramen cleft-** Clefts of lip and palate posterior to the incisive foramen:
   a. Total (Figure 1.7) or partial (Figure 1.8)

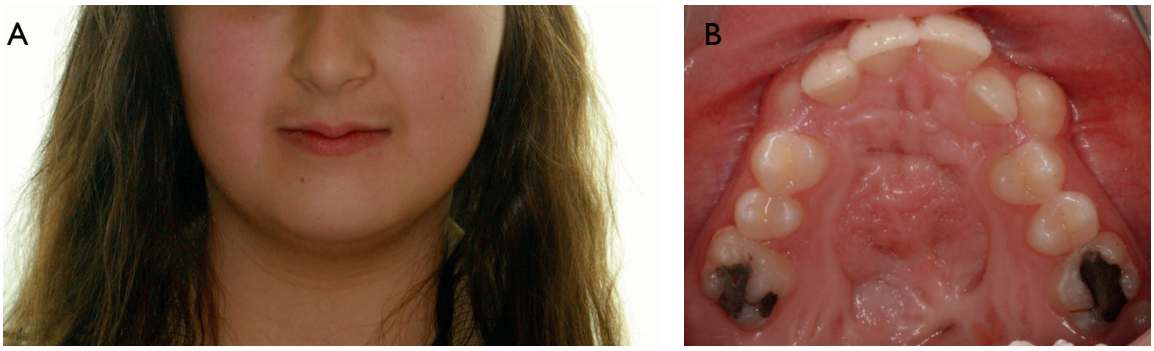

Figure 1.7. Cleft palate: extra-oral photography (A); intra-oral occlusal photography (B).

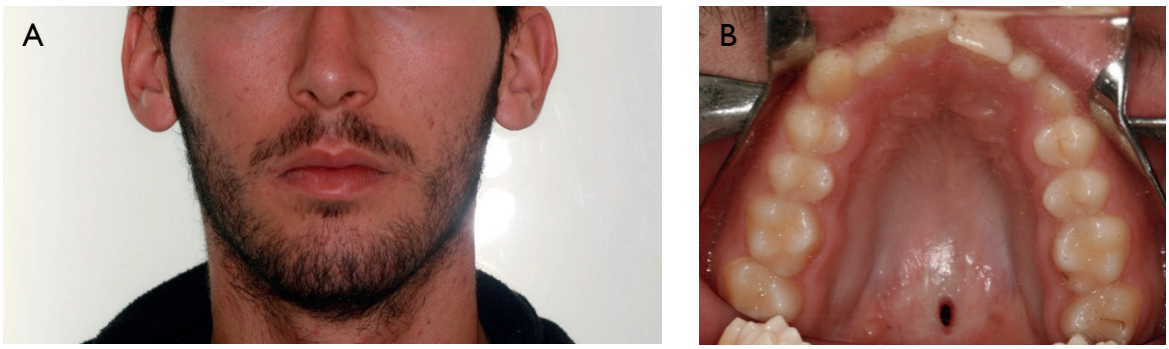

Figure 1.8. Cleft palate: extra-oral photography (A); intra-oral occlusal photography (B).

4. Rare facial fissures- oblique clefts (oro-orbital) (Figure 1.9), transverse clefts (oro-auricular) (Figure 1.10), clefts of the lower lip and others.

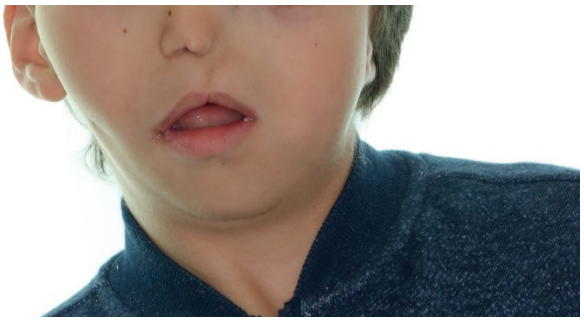

Figure 1.9. Extra-oral photography of oblique clefts (oro-orbital)

Figure 1.10. Extra-oral photography of transverse clefts (oro-auricular)

This classification is still used today because of its simplicity.

5. Clinical Features

CLP leads to several difficulties such as feeding, hearing, speaking, breathing and dentofacial development.[70-72] Dental anomalies are significantly more frequent in CLP patients than in general population, leading to a long-term impact on patient's facial anatomy and self-esteem.[73-75] Studies have shown that there seems to be an association between its severity and the severity of the cleft, referring that some dental anomalies can be clinical markers and define sub-phenotypes of OC.[74-76] Al Jamal et al. showed that bilateral CLP was associated with more hypoplastic teeth, microdontia and dilacerations than unilateral CLP.[76]

The most common dental anomalies in CLP patients are: hypodontia, supernumerary teeth, anomaly of shape and size of the teeth, enamel mineralization and ectopic eruption (Figure 1.11).[77-80]

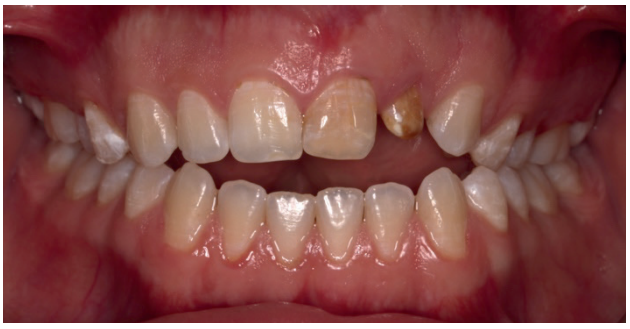

Figure 1.11. Cleft palate: intra-oral occlusal photography with anomaly of shape, size and enamel mineralization of tooth 22.

The lateral maxillary incisor on cleft side was the tooth most frequently missing, following by second premolars on non-cleft quadrants and lateral maxillary incisor on non-cleft side (Figure 1.12).[71,81] Previous studies have suggested different hypotheses for lateral incisors agenesis: odontogenic potential comes from maxillary and medial nasal prominences, so any failure in its fusion may result in agenesis; deficiency of blood supply due to the cleft itself or as a secondary effect of surgery; the bony defect on the cleft.[72,82-83]

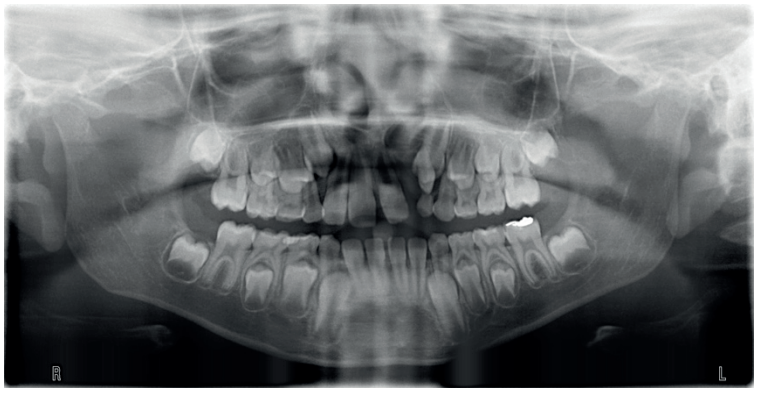

Figure 1.12. Unilateral Cleft lip and palate on the left side: orthopantomography with agenesis of tooth 22.

Additionally, some studies suggested that cleft can modify craniofacial development of the maxilla and mandible.[84-85] However, this remains under debate with several studies proposing the following hypotheses for maxilla: 1) maxilla has an intrinsic growth deficiency in non-syndromic CLP. This hypothesis is based on the fact that CLP patients who have not undergone surgery also show some degree of maxillary retrusion and median facial dysplasia in adulthood; 2) early surgical interventions can produce lip tension and scar tissue surrounding the palate and maxilla, which may induce posterior displacement of the maxilla.[86-91] The magnitude of displacement depends on the amount of tissue defect, skill of the surgeon and surgical protocol.[92-93] Lambrecht et al. described the most frequent maxilla morphology in unilateral CLP: untreated patients frequently presented maxillary protrusion with normal vertical dimension and reduced horizontal dimension; surgical treated patients often adopted a retruded position.[94] Regarding mandibular spatial position, the literature revealed a downward and backward rotation of the mandible associated with a more obtuse gonial angle, showing the vertical growth pattern.[84,95] Furthermore, smaller mandibular ramus length was also reported.[95-96]

The presence of dental anomalies and abnormal craniofacial development can lead to the development of malocclusion such as crossbite (anterior or posterior), open bite, skeletal Class III and crowding.[97-98] According to Vetorre et al., the most common malocclusion in CLP patients between 6 and 12 years is anterior crossbite (60.7%) and the upper crowding in the anterior segment (69.6%).[97] The transversal growth of maxilla can be affected by cleft palate closure surgery and the presence of agenesis in the cleft. Thus, crossbite in CLP patients is due to maxillary arch constriction, lingual crown tipping of maxillary anterior teeth and palatal tipping of maxillary posterior teeth (Figure 1.13).[99] The frequencies of skeletal Class I, II, and III malocclusions in CLP patients were 18.5, 8.8 and 72.7, respectively.[98]

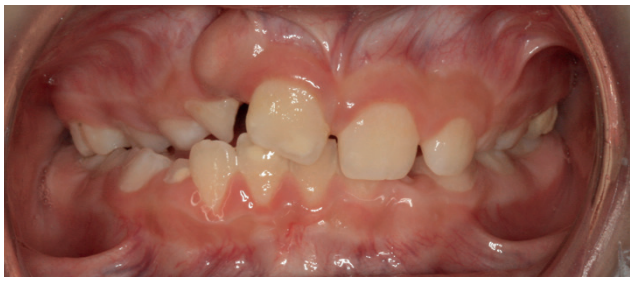

Figure 1.13. Unilateral Cleft lip and palate with crossbite on the right side

6. Primary Prevention

The clinical characteristics of CLP patients require a series of treatments from birth to adulthood, which is a burden on individuals, families, and healthcare systems.[70,100]

In some countries all over the world, healthcare systems cannot afford treatment for cleft lip and palate. Identification of strategies to modify risk factors for non-syndromic CLP is the first step toward primary prevention.[13] The following are key perspectives from the World Health Organization meetings on International Collaborative Research on Craniofacial Anomalies on primary prevention strategies: 1) identify environmental and behavioural factors; 2) summarize the evidence of specific maternal nutritional factors in the etiology of orofacial clefts; 3) create a consensus regarding the role of nutritional supplementation; 4) definition of the design of prevention trials regarding their ethical, legal, social and financial implications; 5) make recommendations for new researches with international collaborative studies.[101]

Several efforts have been made to understand the etiology of CLP in order to prevent its occurrence namely, manipulation of maternal lifestyle, nutritional advice (eg multivitamin and mineral supplements), prevention of certain drugs and awareness of others risk factors (eg occupational, social and demographic).[25-26,30-31] As strong evidence in some of prevention factors is still lacking, the next reasonable step for research might be the observational studies.[13] These trials will allow to incorporate their findings into health promotion programs for women of reproductive age.

7. Management of CLP patients

Management of CLP patients requires an interdisciplinary team of specialists to achieve normal speech, hear and occlusion with a normal facial appearance and psychological well-being.[102] The timing and sequencing of treatment should be a result of the coordinated decision of a multidisciplinary team focusing on a patient-centered care and family's needs approach.[103]

A team approach for providing care has been developed since the establishment of the American Cleft Palate Association (ACPA) in 1943.[104] Since then, the treatment of CLP patients have improved but some patients still receive inadequate care due to diagnostic errors, undiagnosed problems, unnecessary or inappropriate procedures and inadequate timed treatment.[105] The interdisciplinary team must assure the quality of treatment with the most efficient use of time and resources. Several practitioners should include the interdisciplinary team (figure 1.14) but not be limited to[105-106]:

- Nursing Care:
  - Services include feeding assessment, nutritional advice, interventional teaching and growth assessment.
- Surgery- Multiple operative procedures were performed from infancy to adulthood:
  - Primary cleft lip surgery is usually performed until the twelfth month of life, while cleft palate closure is typically performed until the eighteenth month.
  - Patients with microtia can be submitted to surgical reconstruction of the middle ear, auditory canal and external ear.
  - In adulthood, rhinoplasty and orthognathic surgery may be indicated.
- Otolaryngology and Audiology:
  - A record of the newborn's hearing should be obtained at birth.
  - Audiological evaluations should begin at approximately nine months of age with behavioral and physiologic testing.
  - CLP patients can lead with airway obstruction, dysfunction in the middle ear, otitis media and other pathologies that requires otolaryngology intervention.

- Genetics- Genetic testing helps the diagnosis and inform about the prognosis, especially in syndromic patients who do not fully express the clinical features in the first years of life.
- Pediatrics:
  - Monitor children for growth failure, delayed development, abuse and neglect, or other significant health concerns.
- Speech and language therapists:
  - Evaluations should begin before palate closure and include language development, articulation development, and assessment of resonance and voice.
- Psychology:
  - Psychosocial screening should begin in infancy and include several evaluations, namely, child-parental competence, parent-child relationships, child management skills and emotional and behavioral adjustment.
- Dentistry:
  - Dental services occurs from infancy to adulthood and may include: preventive dental care, restorative dentistry, infant orthopaedics, orthodontic treatment and prosthetic treatment.

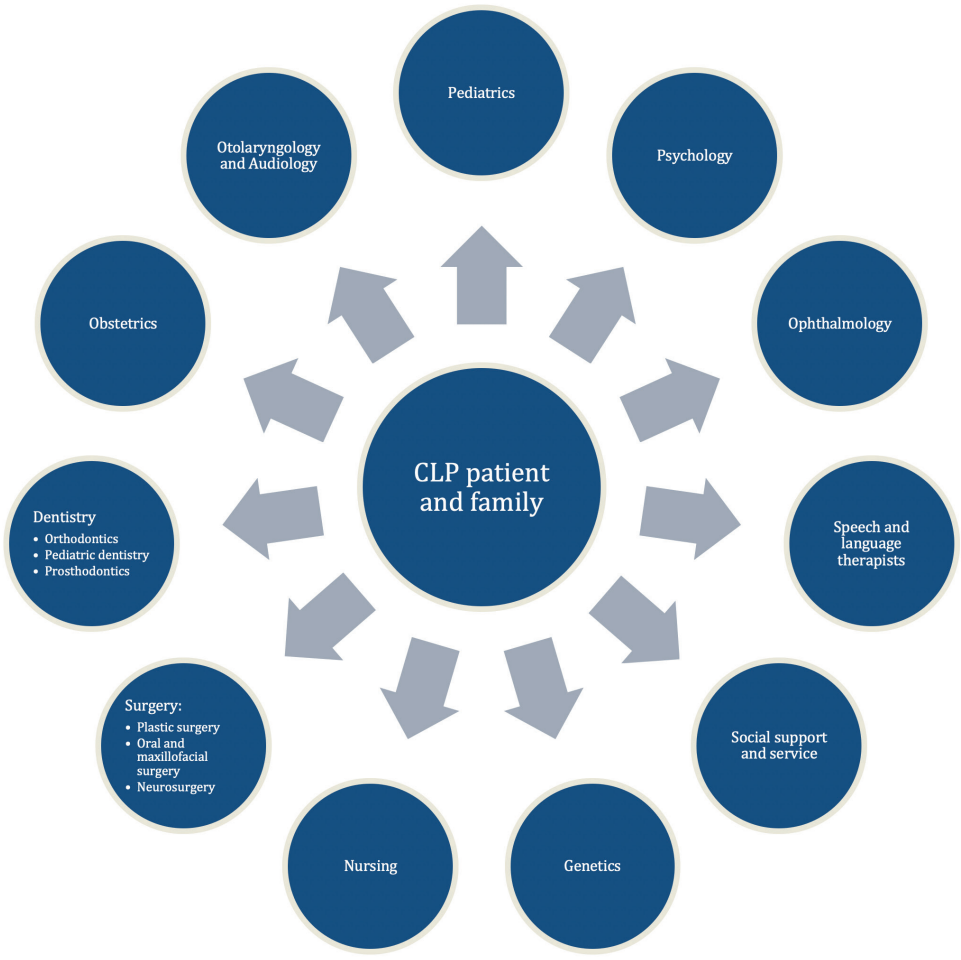

Figure 1.14. Multidisciplinary team in CLP patients. Adapted from Wyszynski.[15]

8. Orthodontic treatment in CLP patients

The orthodontists´ role in the treatment of CLP patients begins shortly after birth to adulthood. Timing and sequencing of orthodontic care will be described for four categories according to age and dental development: birth to 2 years, 2 to 6 years, 7 to 12 years, 13 years to adulthood.

Birth to 2 years

The goal of neonatal treatment is the closure of lip and/or palate, which can be preceded by presurgical maxillary orthopaedics (PSO) that aims to realign the maxillary segments excluding the tongue from the cleft. However, since its introduction by McNeil in the 1950s, several modifications and appliances have been suggested with controversial outcomes.[107-108] PSO can be allocated into three categories: 1) passive-realign of segments during growth by grinding away material in some areas of the plate; 2) semi-active- the plate reorients the maxillary segments in the predetermined direction; extra-oral strapping can be used in this protocol; 3) active appliances- the appliance applies a force to the maxillary segments in the predetermined direction using a delivery system such as screws or springs.[108]

Studies reported that the modelling of nasal cartilage should be performed in the first 6 weeks after birth due to the high levels of maternal estrogen in the fetal circulation. These levels promote an increase in elasticity of cartilage, ligament, and the connective tissue in the newborn.[109-110] Subsequently, nasoalveolar molding (NAM) was described by Grayson with the aim of realigning maxillary segments and modifying columellar length and nasoalar form due to the addition of extraoral projections (nasal stents) (figure 1.15).[111]

Figure 1.15. Nasoalveolar molding with one nasal stent

Treatment started before a month of age shows better results in nasal symmetry, with long-term stable results.[111-113] Although based on the literature, definitive conclusions about the effectiveness of nasoalveolar molding cannot be drawn. The suggested advantages and opponents views of NAM are shown in Table 1.2.[114-127] LevyBercowski et al. summarised the complications relating to NAM namely, mucosal ulceration, bleeding, tissue fungal infections and asymmetrical arch form.[128]

Table 1.2. Suggested advantages and opponents views of NAM[114-127]

	Suggested advantages	Opponents views
Surgery	Cleft reduction promotes less lip tension, which benefits wound healing postoperatively[114]	
	Allow symmetrical arch form[114]	Restricts maxillary development as a result of the molding process[115]
	Improves position of alar base[116-117]	Over the years, the differences between patients that undergo PSO or not are smaller[118]
	Prevents initial collapse after surgery[119]	
	Pull the premaxilla back into the normal position in bilateral cleft patients[117]	
Growth	Reduce the width of the alveolar and palatal cleft[120]	Restricts maxillary growth due to mold process[115]
	Reduction of tongue interference in the cleft may encourage the normal growth of the palatal shelves[119]	
Function	Improve speech because improve physiological function and position of the tongue (prevents twisting and dorsal position of the tongue in the cleft)[121]	The delay in palate closure decrease speech[122]
	Improve feeding[119]	Do not improve swallowing or feeding efficiency or general body growth[123-124]
	Improve nose breathing and decrease nasal regurgitation[119]	
	Positive psychological effect on the parents[119]	No significant improvement in parents' satisfaction[121]
Cost-effectiveness	Tridimensional technology can decrease the time of treatment by reducing the clinical chair time and the cost of appliance[125] NAM may eliminate the need for a second operative intervention, decreasing total hospitalization time and cost[126-127]	The overall cost of treatment due to frequent visits and fabrication method[121]

Primary lip repair is performed after the effect of NAM is achieved, restoring the continuity of the mucosa, skin and underlying mucosa. The alignment of the maxillary segments obtained by the PSO is considered similar to those produced by surgical lip adhesion, which is a partial lip repair procedure in wide clefts.[129] Lip closure is usually performed when the children is 3 to 6 months of age while the closure of the palate occurs between 12 months to 2 years of age. Timing of palate closure is related with the development of the speech and language skills.[103] Figure 1.16 presents a case report of NAM treated at the Institute of Orthodontics, Faculty of Medicine, University of Coimbra (FMUC).

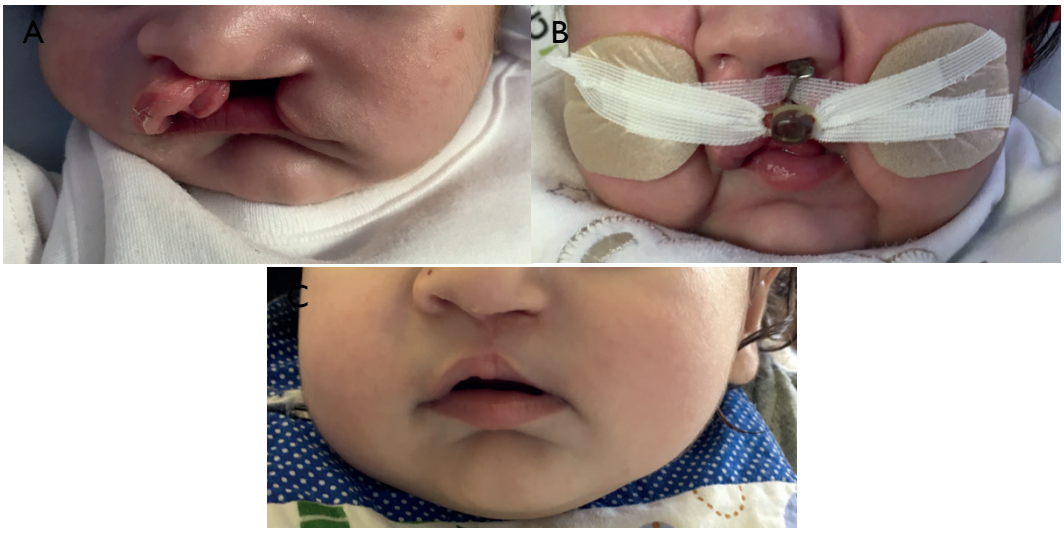

Figure 1.16. Case report of NAM: A) initial extra-oral photography: B) preoperative photography with the NAM; C) two weeks after lip closure

Deciduous dentition: 2 to 6 years

Orthodontist should closely monitor the dental development as cleft can disrupt the development of the dentition and the chronology of eruption.[130] Establishment of the deciduous dentition permits to identify skeletal and dental components, allowing to diagnose developing malocclusion. If craniofacial development is normal, orthodontic consultation should be performed annually to monitor facial growth and discuss future treatment. Orthodontist should consider several factors to determine when to initiate the treatment, namely, children´s cooperation, severity of malocclusion, timing of secondary bone graft and the need for future orthodontic treatment.[103] After considering all these factors, some orthodontic interventions can be delayed until the mixed dentition.

Malocclusion may be present due to primary lip and palate surgeries that recover the esthetics and function but can restrict maxillary growth in the anteroposterior and transverse plane.[131-132]

Antero posterior discrepancy typically leads to the development of an anterior crossbite and a Class III malocclusion.[133] Class III malocclusion is evident in younger ages and progressively increases, having been reported in approximately 70% of CLP patients with 12 years.[134-135] Early orthopaedic

correction through the facemask is the most commonly used treatment. This treatment promotes protraction of the maxilla, downward and backward rotation of the mandible, retroclination of the lower incisors and proclination of the upper incisors.[136] Maxillary retrusion is more frequent in unilateral CLP than bilateral CLP, since bilateral CLP usually have a prominent premaxilla that becomes less protrusive over the time.[131,137]

Regarding the effects of facemask, protraction is more effective during the deciduous dentition period because reduces dentoalveolar compensations such as proclination of upper incisors in the permanent dentition.[138] Unilateral CLP has a skeletal/dentoalveolar effect of 45%/55% while bilateral CLP has an effect of 10%/90%.[137] Tindlund found better skeletal response when protraction began at 6 years (mean age 6.3) due to the patency of circummaxillary sutures at this age.[139] Recently, a meta-analysis summarised the major effects of facemask in unilateral CLP patients, who state that facemask increase SNA (2.12° , 95% CI), ANB (4.17°, 95% CI) and decrease SNB angle (−1.94°, 95% CI).[140] Figure 1.17 shows the patient lateral cephalograms and the lateral profile at the beginning and after treatment with facemask.

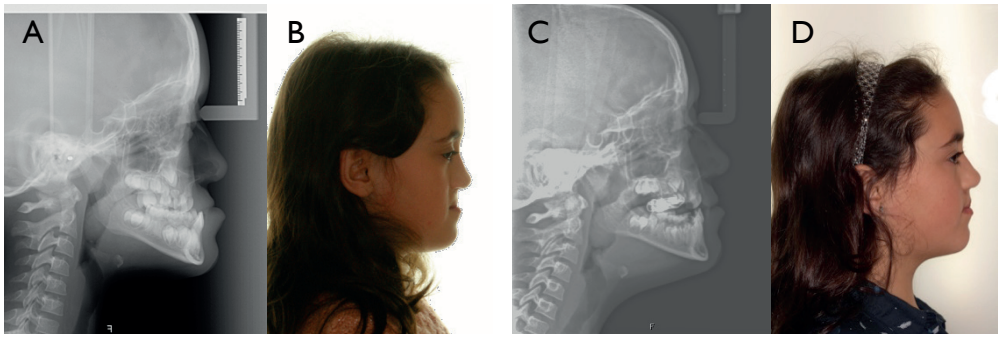

Figure 1.17. Case report of facemask: A) initial lateral cephalogram; B) initial lateral profile; C) lateral cephalogram after facemask; D) lateral profile after facemask.

The management of Class III maloclusion is challenging, because after protraction the initial growth pattern reappears, with changes in the position of the mandible while maxilla remains stable. Despite that, patients that undergo facemask have an improvement in occlusion, creating better conditions for craniofacial development.[138,141]

Mixed Dentition: 7 to 12 years

The mixed dentition starts with the eruption of the first permanent molars and incisors. At this stage, CLP patients may require secondary bone grafting, which offers the following benefits: 1) bone support for unerupted teeth adjacent to the cleft; 2) closure of oronasal fistulas; 3) support alar bases, which helps nasal and lip symmetry; 4) stabilise the cleft maxillary segments and eliminate the notched alveolar ridge; 5) stabilise and minimize the protrusion of premaxilla in the bilateral CLP.[103,108] Since its introduction by Boyne and Sands in 1972, bone graft using cancellous bone from the iliac crest remains the gold standard until today (Figure 1.18).[142-143] The timing of the bone graft is dependent on dental development; usually it is performed when canine has one-half to two-thirds root formed,

which typically occurs between 9 to 11 years. Rarely, it can be performed prior the eruption of the lateral incisor in order to improve its outcome.[143] The success of alveolar bone grafting requires pre-surgical orthodontics that includes normally: correction of maxillary discrepancies, incisor alignment and control of the canine eruption.

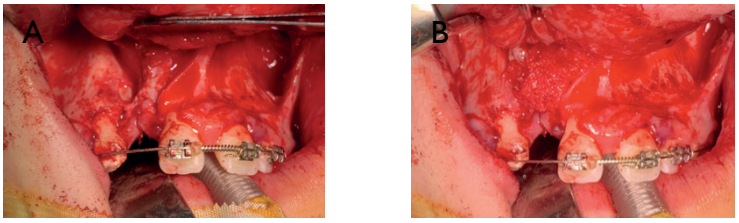

Figure 1.18. Alveolar bone grafting: A) preparation of flat recipient bed; B) bone graft using cancellous bone from the iliac crest on cleft

The maxillary expansion is commonly used as part of sequential treatment for cleft patients. The main goals of maxillary expansion are to correct the transverse discrepancy, establish the maxillary arch form, make a room for bone graft by widening the alveolar cleft and improve access to the alveolar bone graft area.[144] Several studies recommend that maxillary expansion should be initiated at least 6 months before alveolar bone graft surgery.[103,144] However, more recently, studies reported that maxillary expansion after secondary bone graft does not compromise the integrity of the grafted area in selected cases, yet this topic needs further investigation.[145-146]

The effects of maxillary expansion on non-CLP patients are well-established, while the biomechanical effects in CLP patients appears to be different.[147-148] Pan et al. shows that CLP patients had an asymmetric expansion with dispersed stress distribution around the lateral maxilla buttress and outboard of orbit.[148] Maxillary expansion is usually carried out during the mixed dentition because the mid-palatal suture becomes more inter-digitated with age and therefore heavier forces are needed in older adolescents and adults.[149]

Regarding the expansion protocol, slow maxillary expansion was suggested instead rapid maxillary expansion based on the following assumptions: a) palatal suture system is disturbed and either irregular or absent, which increases the response to orthopedic forces; b) rapid maxillary expansion had some disadvantages such as: requires patient cooperation in activation, open bite, micro trauma of the temporomandibular joint, root resorption, tissue impingement and pain; c) slow maxillary expansion produce less tissue resistance around the circummaxillary structures, which improve bone formation in the intermaxillary suture.[150] These results are corroborated by Almeida et al. that showed that both expansion protocols can be similarly indicated to correct maxillary transverse discrepancy in bilateral CLP patients.[151] Furthermore, the anatomical variability of the upper arch has led to the development of several designs of maxillary expanders, depending on whether the constricted region of the maxilla is anterior, posterior or both. The inverted mini-hyrax promotes more expansion in the anterior region of the maxilla and does not cause as much buccal tipping of the supporting teeth as the fan-type. Both hyrax and quad-helix expanders can be used when anterior and posterior maxillary expansions are needed (Figure 1.19).[150,152]

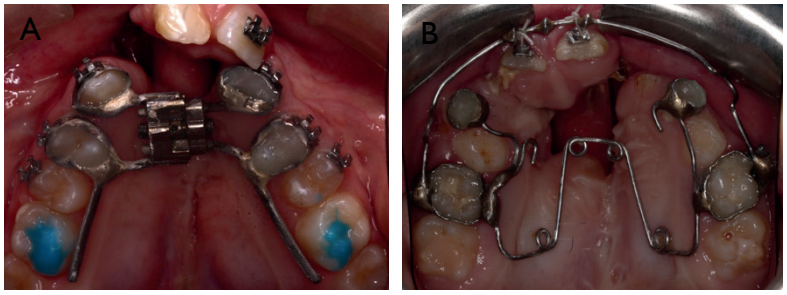

Figure 1.19. Maxillary expansion appliances: A) mini-hyrax; B) quad-helix

Alignment of the upper dental arch may be necessary because incisors often erupt rotated, tipped and retroclined, placing them in a crossbite.[153] Activation of the appliances can start before the bone graft in order to achieve optimal dental space, an aesthetic incisor relationship to the facial midline and immobilize the premaxilla segment in bilateral CLP.[103,153] The orthodontic treatment should be restarted 3 months after the secondary alveolar bone grafting in order to move the roots of the teeth adjacent to the cleft into the grafted bone, improving the consolidation of the alveolar bone and the height of the crest.[154] If canine does not erupt spontaneously through the grafted bone, orthodontic traction may be necessary.[103,153]

Permanent Dentition: 13 to adulthood

After bone grafting, if craniofacial development occurs favourably, fixed orthodontic appliances continue to re-establish facial esthetics and proper function.[155] However, if a skeletal discrepancy develops, the orthodontist should consider the previous surgical history, severity of skeletal discrepancy and the cessation of craniofacial growth to plan orthodontic treatment.[108,156] The best method to determine the end point of craniofacial growth is through longitudinal cephalometric data with a minimum interval of 6 months.[108]

Camouflage treatment can be carried out in non-growth patients with mild skeletal Class III relationship and minimal esthetics concern by increasing the vertical dimension that promotes the rotation of the mandible backward and downward.[103] The decision for camouflage treatment should be taken with care because earlier treatment can produce suboptimal results. Thus, this treatment option should take into account the patient´s desires, occlusion, esthetics and biological limits.[103,156]

Patients with more severe discrepancies may need a combined surgical and orthodontics treatment to obtain a normal occlusion, and good support for the nose and upper lip.[157] Presurgical orthodontics is required to decompensate maxillary and mandibular teeth in relation to their skeletal bases.[156] To prevent deterioration of soft palate function, velopharyngeal function and speech ability must be assessed prior planing the displacement of the maxilla.[158] The conventional surgical procedure for correcting maxillary hypoplasia is a Le Fort I osteotomy. The surgical relapse reported was 10% and 25-50% in non-cleft and CLP patients, respectively.[159-160] Alternately, distraction osteogenesis in orthognathic surgery has been suggested as an effective approach for the treatment of cleft lip and palate. [161-162] Distraction can be performed with internal or external distraction devices (Figure 1.20).

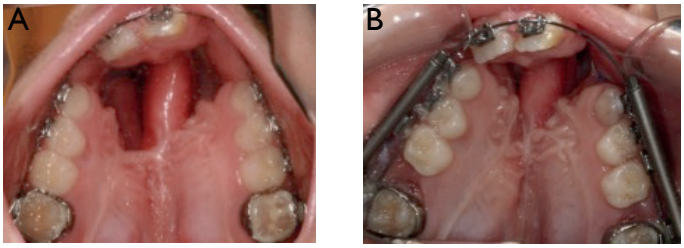

Figure 1.20. Bilateral cleft lip and palate: A) occlusal photograph before distraction; B) Internal distraction device- occlusal photograph after two weeks of distraction

The clinical indications regarding ideal age, surgical technique, distraction device, and retention remain controversial.[163] Studies hypothesised that distraction osteogenesis has less relapse than Le Fort I osteotomy because the combination of the gradual movement of the maxilla and the resistance of the distracter may reduce the influence of scarred tissues and muscles which promotes the backward and upward of maxilla.[160,162,164] Some systematic reviews have attempted to compare the effectiveness of distraction osteogenesis and orthognathic surgery, concluding that there is insufficient evidence between both methods. The main findings of these systematic reviews show that: 1) both surgical osteotomy methods can produce significant hard and soft tissue improvements; 2) relapse is lower in the distraction osteogenesis five years after surgery; 3) no statistically significant differences regarding speech and velopharyngeal function were found; 4) clinical morbidities are similar.[158,165]

After active orthodontic treatment, retention is crucial especially in CLP patients due to their clinical features such as dental anomalies, transverse arch discrepancies and defects in hard and soft tissue. The Hawley retainer is the mostly prescribed appliance in CLP patients.[166]

After establishing the occlusion, orthodontist should discuss the treatment plan with the prosthodontics in order to replace missing teeth, restore hypoplasic incisors or modify the shape of the canine when it replaces lateral incisor.[104]

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II

Chapter II

Parental risk factors and child birth data in a matched year and sex group cleft population: a case-control study

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1. Introduction

Orofacial clefts (OC) are one of the most common craniofacial malformations, with an international prevalence in newborns of 14 per 10,000 live births worldwide.[1-2] The prevalence of OC has been increasing over the years, perhaps as a result of improved surgical techniques, neonatal care resulting in reduced postnatal morbidity and mortality, more accurate documentation, and environmental factors such as smoking consumption.[2-4] According to the European Network for Epidemiological Surveillance of Congenital Anomalies (EUROCAT) report, the prevalence in 26 European countries was 14.5 per 10,000 births between 2011 and 2018.[5]

The etiology of OC is not completely known but several genetic and environmental risk factors have been identified. Previous studies have clarified the importance of epidemiologic knowledge, especially in 1) assessing the burden of OC in order to plan public health resources; 2) knowing the causes of OC, which stimulates research on primary prevention and treatments to improve the quality of care.[6] The genetic component plays an important role in the formation of isolated cleft. Some of the best-supported genes and genetic loci in the literature are IRF6, ch8q24, VAX1, and PAX7.[7-8] Concerning environmental risk factors, previous researches, which have shown consistency, have proposed smoking consumption during pregnancy and gestational diabetes as maternal risk factors.[4,9-10] Other associations have been reported with less consistency, namely alcohol consumption during pregnancy, intrapartium interval, maternal and paternal age, and nutritional deficiencies (folic acid, vitamin A and B12, and zinc).[11-18] Furthermore, both extremes of maternal body mass index (BMI) have been associated with birth defects, but with controversial results in the literature.[10,19-21] Recently, Kutbi et al. showed in a larger population-based study that obesity was associated with an increased risk of OC but no conclusion was drawn about maternal underweight.[21]

Even though there are several studies about OC etiological factors in the literature, there is a gap regarding neonatal characteristics. Several studies showed that OC children are lighter and smaller than healthy controls but with inconsistent results.[22-23] Despite the growing literature about cleft etiology, the main limitation of these studies is the recruitment process, since there is often

heterogeneity in relation to the sociodemographic factors, sex and age of patients evaluated. This approach is expected to produce more bias since it is already known that some of these factors are determinants of laterality and side of the cleft, such as sex.[24] Furthermore, the studies published on cleft birth data are focused on weight, leaving aside some anthropometric measurements (eg, length and head circumference at birth) that are used in the evaluation of intrauterine growth deviations, overall health of the child, and early and long-term growth.[22,25-26]

This study aims to evaluate the effect of possible parental related influencing factors on the development of cleft lip and/or palate as well as the differences in birth data. Therefore, this study used child recruitment with standardized methods in order to obtain a homogeneous sample regarding age, sex, and sociodemographic factors. Moreover, length and head circumference at birth will be measured in addition to birth weight. The purpose of this study was to test the hypothesis that the risk of having a cleft child increased with parental age or maternal BMI, and also to compare the differences in birth data (birth weight, birth length, and head circumference at birth) between cleft children and healthy controls (non-cleft group).

2. Materials and Methods

Study Design

This retrospective case-control study was approved by the Ethics Committee of the Faculty of Medicine of University of Coimbra (Reference: CE-072/2020) (Appendix 2.1). The study was handled in accordance with the Declaration of Helsinki.

Data Collection Procedure

The study group included parents from children (born between 1995–2015), with non-syndromic OC, being rehabilitated at the Institute of Orthodontics, Faculty of Medicine, University of Coimbra (FMUC), regardless of sex or place of birth. The control group included individuals without clinical alterations or craniofacial defects. To minimize bias, controls were matched according to year of birth and sex. Both groups had similar socio-economic backgrounds.

Data were collected through two sources: (1) medically recorded data—parents were requested to send the pre-natal care logbook and all medical record during the pregnancy; (2) maternal self-reported information.

A structured questionnaire was also distributed to the parents of both groups after the clinical visit, avoiding any harm to the patients (Appendix 2.2). The questionnaires were deployed always by the same examiner (I.F.) after being properly trained for the activity. Before fulfilling the questionnaire, parents were asked to read the enclosed list as a memory aid with the most common maternal diseases during pregnancy and pregnancy supplements. From both groups, the parents who did not consent to participate in the study or who did not answer the questionnaire sufficiently, parents with consanguineous marriages, and children with syndromic OC were excluded.

The questionnaire included retrospective parent and patient information. Variables were divided in three groups:

- Sociodemographic—age of parents and child; child's sex.
- Obstetrical data—alcohol intake, smoking, drugs, and pregnancy supplements used, diseases diagnosed before and during pregnancy and exposure to carbon monoxide, recorded by gestational month; early pregnancy body mass index.
- Child birth data—presence and phenotype of cleft, birth weight, birth length, and head circumference at birth.

After matching the control group to the OC group, the final sample consisted of 266 individuals (Figure 2.1). All participants gave their written informed consent for secondary use of their records.

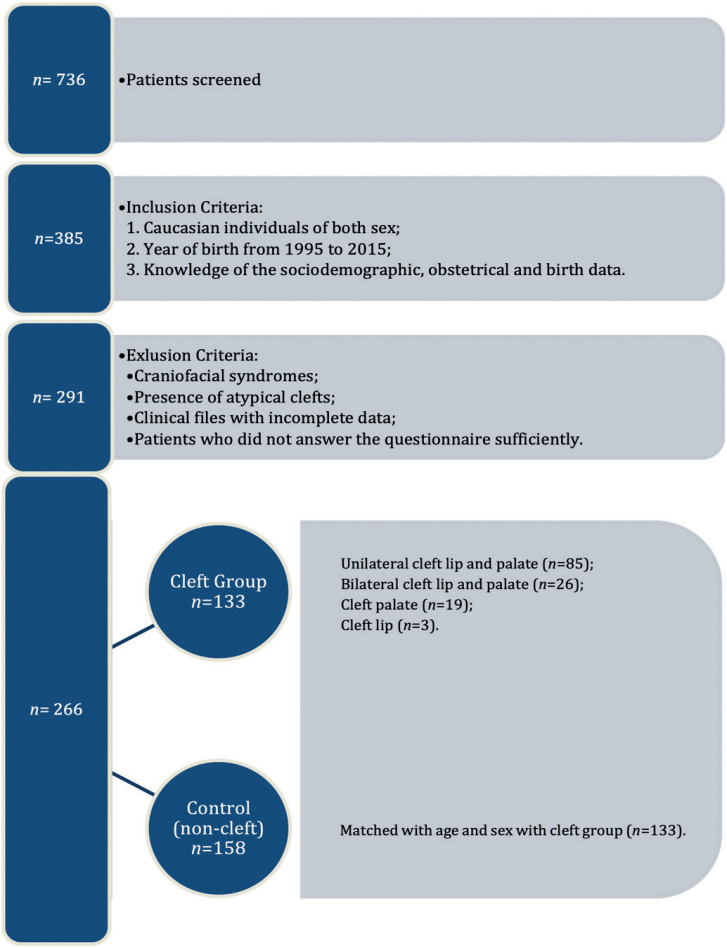

Figure 2.1. Flow chart of the inclusion and exclusion criteria.

Statistical Analysis

All analyses were performed using the Statistical Package for the Social Sciences, version 26.0 for Windows (SPSS Inc., Chicago, IL, USA). The significance level chosen was 0.05.

Descriptive statistics for the quantitative variables were obtained using mean, standard deviation values, and 75th and 25th percentiles. Categorical variables were expressed as absolute and relative frequency.

The comparison between the groups was performed using Fisher's exact test for sex (nominal variable), and for the quantitative variables, the Mann-Whitney test was used when a violation of the normality assumption was verified through the Shapiro-Wilk test.

To assess the risk factors, a logistic model was adjusted using as independent variables parental age, maternal BMI, and birth data (weight, length, and head circumference at birth). The logistic model was evaluated using the Naguelkerke's R2 and by measuring the area under the ROC curve. The null hypothesis of the statistical analysis (the logistic regression) is that no risk factors exist.

Comparison between the different phenotypes of cleft was performed using the ANOVA test or the Kruskal-Wallis test when the normality assumption was not observed. Tukey's post-hoc tests were performed, after verifying the significant ANOVA test.

3. Results

In total, 266 eligible cases were identified in the FMUC between 1995 and 2015, among them 133 were orofacial cleft and 133 were control group.

Table 2.1 presents descriptive statistics concerning sex distribution, parental age, maternal BMI, and birth data of the subjects studied. The distribution of age and sex is homogenous (p = 1.00) between groups (Control vs. Orofacial Cleft).

Table 2.1. Descriptive analysis by each group.

Variables	Control (133)	Orofacial Cleft (133)	p
Sex (M/F)	85/48 (63.9%/36.1%)	85/48 (63.9%/36.1%)	1.000 #
Maternal age (years) [a]	31 (4.2) 28.0/34.0	28 (5.6) 25.0/32.0	<0.001 §
Paternal age (years) [a]	33 (4.9) 29.0/36.0	31 (6.3) 27.0/35.0	0.016 §
Maternal BMI (kg/m^2) [a]	23 (3.9) 20.8/25.1	25 (4.3) 21.9/28.3	<0.001 §
Birth length (cm) [a]	49 (2.1) 48.0/50.0	48 (2.9) 47.0/50.0	0.519 §
Birth weight (kg) [a]	3 (0.5) 3.0/3.6	3 (0.5) 2.8/3.4	0.033 §
Birth head circumference (cm) [a]	35 (2.2) 33.5/35.0	34 (2.3) 33.5/35.0	0.457 §

[a] mean (standard deviation) 25–75 percentiles; # Fisher's exact test; § Mann-Whitney, M-male, F-female, BMI- body mass index.

Three independent variables (maternal age, maternal BMI, and child birth weight) found statistical significance using the logistic model (p < 0.001). The model obtained explains about 17% of the variance ($R^2_{Naguelkerke}$ = 0.169) and presents an accuracy of 63.2% which is compared with 50% obtained in the null model. Table 2.2 shows the regression coefficients obtained with the corresponding adjusted odds ratios (OR) and their confidence intervals (CI).

Table 2.2. The regression coefficients for maternal age, maternal BMI, and weight at birth.

Variables	B	p	OR	CI95%
Maternal age (years)	−0.102	<0.001	0.903	[0.856; 0.953]
Maternal BMI (kg/m^2)	0.131	<0.001	1.140	[1.068; 1.216]
Birth weight (kg)	−0.833	0.003	0.435	[0.253; 0.746]
	2.477	0.062	11.904	

B – regression coefficient; OR – odds ratio; CI – confidence interval.

Regarding parental related factors, it was found that 1) for each maternal year increase, the probability of having a cleft child decreases 0.9 (OR=0.903) and 2) for each BMI unit (kg/m^2) increase, the probability of having a cleft child increases 1.14 (OR=1.14). The child-birth data showed that for each mass unit (kg) increase at birth, the probability of having a cleft child decreases 0.4 (OR=0.435). The area under the ROC curve for the probabilities obtained by the logistic model was 0.699 (IC95% [0.637; 0.761], p < 0.001).

No association between maternal age and BMI index on the risk of having a child with a cleft was found (p = 0.573).

The final sample of OC group consisted of 85 unilateral cleft lip and palate, 26 bilateral cleft lip and palate, 19 cleft palate, and 3 cleft lip. Table 2.3 presents descriptive statistics concerning phenotype of cleft. It was found that maternal BMI affects the risk of having cleft in the four clefts types (p = 0.023) with a statistical difference in cleft lip (Table 2.4).

Table 2.3. Descriptive statistics concerning phenotype of cleft.

Variables	Unilateral Cleft Lip and Palate (n = 85)	Bilateral Cleft Lip and Palate (n = 26)	Cleft Palate (n = 19)	Cleft Lip (n = 3)	p
Maternal age (years) [a]	28 (5.4) 25.0/31.0	28 (5.8) 24.0/31.0	30 (5.8) 26.0/37.0	30 (9.1) 23.0/40.0	0.4271 [§]
Paternal age (years) [a]	31 (6.2) 27.0/34.0	32 (6.9) 28.0/34.0	34 (5.4) 29.0/38.0	27 (3.2) 25.0/31.0	0.121 [£]
Maternal BMI (kg/m^2) [a]	25 (4.2) 22.3/27.6	25 (5.0) 20.3/29.1	25 (3.6) 21.8/26.4	33 (1.7) 30.8/34.2	0.023 [£]
Birth length (cm) [a]	48 (2.7) 47.0/50.0	48 (2.6) 47.0/50.0	49 (4.3) 48.0/51.0	48 (1.2) 47.0/49.0	0.452 [§]
Birth weight (kg) [a]	3 (0.5) 2.8/3.4	3 (0.5) 2.9/3.5	3 (0.4) 2.9/3.4	3 (0.7) 2.2/3.6	0.505 [£]
Birth head circumference (cm) [a]	34 (1.7) 33.0/35.0	34 (1.2) 33.5/35.0	36 (4.3) 33.5/36.0	33 (2.6) 30.0/35.0	0.702 [§]

[a] mean (standard deviation) 25–75 percentiles; [§] Kruskal-Wallis; [£] ANOVA.

Table 2.4. Correlation between maternal BMI and cleft phenotype.

Phenotype of Cleft	p
Unilateral cleft lip and palate vs. bilateral cleft lip and palate	p = 0.990
Unilateral cleft lip and palate vs. cleft palate	p = 0.947
Unilateral cleft lip and palate vs. cleft lip	p = 0.016
Bilateral cleft lip and palate vs. cleft palate	p = 0.995
Bilateral cleft lip and palate vs. cleft lip	p = 0.016
Cleft palate vs. cleft lip	p = 0.014

4. Discussion

The purpose of this study was to evaluate the effect of parental age and mothers' BMI on the risk of having a child with OC. Possible interaction of mother risk factors is also investigated. Moreover, birth data of cleft children were analyzed to assess the differences between patients with OC and healthy controls. Of the six study variables, three variables (maternal age, maternal body mass index, and birth weight) found statistically significant differences using the logistic model ($p < 0.001$).

The current study suggests that for each maternal year increase, the probability of having a cleft child decreases 0.9 (OR=0.903) but no statistical differences were found regarding paternal age. The association between the risk of having cleft and parental age does not have a consensus in the literature.[27-29] Herkrath et al. suggested that mothers 35 years of age or older and parents 40 years of age or older had an increased risk of 20% and 58% of having a child with a cleft, respectively.[27] In contrast, Carvalho et al. did not find an association between maternal age and orofacial clefts ($p = 0.747$) in all age groups study, including mothers 35 years of age or older.[28] Nevertheless, some studies reported that the risk of having a cleft is also related to the interaction of the age of both parents.[30-31] Berg et al. showed that the risk of having cleft lip only increases when the age of both parents was high.[31]

A positive association between BMI and orofacial cleft was also found: for each BMI unit (kg/m^2) increase, the probability of having a cleft child increases 1.14 (OR=1.14). These findings are consistent with the results of three meta-analyses that found an association between overweight women and orofacial clefts with a similar odds ratio for cleft palate and for unilateral cleft lip and palate.[32-34] However, the main limitation of these studies is that they did not analyze the interaction of BMI in cleft lip and bilateral cleft lip and palate. The present study proved that BMI affects the risk of having cleft in the four cleft phenotypes ($p = 0.023$) with a statistical difference in cleft lip (Table 2.4). A cohort study from United Kingdom, investigating the association between BMI and the majority of structural congenital anomalies, included three types of cleft (cleft lip; cleft lip and palate; and cleft palate) and found that only the cleft lip shows a statistical difference (aOR=3.71, 95% CI: 1.05, 13.10; $p = 0.04$).[35] The mechanism that explains how obesity acts as teratogenic factor has not

been fully elucidated. Still, some authors suggested a possible explanation based on the relationship between obesity and gestational diabetes since hyperglycemia can modify the fetal expression of developmental genes, such as bone morphogenetic protein 4.[34]

Despite the average associations regarding maternal age and maternal BMI, no exploration of possible interaction in both risk factors has been done. Thus, this is the first study that intends to verify this relation, revealing that no association can be established between these two factors ($p = 0.573$). This result is unexpected because ageing is associated with an increase in abdominal white adipose tissue and fat deposition in skeletal muscle, which significantly affect insulin sensitivity.[36] Moreover, changes in gametes through life due to environmental exposures or chromosomal alterations and increased permeability to teratogenic agents in the placenta in older mothers may also increase the risk of having a child with OC.[37]

Regarding child birth data (weight, length, and head circumference), all variables presented a lower mean in the cleft group compared with non-cleft individuals. However, only birth weight found statistical differences using the logistic model ($p < 0.001$), suggesting that for each increase in mass unit (kg) at birth, the probability of having a cleft decreases 0.4 (OR=0.435). This finding is consistent with the Wyszynski et al. study, which verified that cleft palate patients with or without lip involvement had slightly lower birth weight (mean difference 159 and 197, respectively).[23] Becker et al. also showed that patients with cleft lip with or without palate involvement were lighter and shorter than control subjects.[22] These authors attempted to hypothesize several explanations for the lower weight in cleft patients at birth: 1) incomplete development of facial tissues; 2) environment factors associated with the risk of having cleft (such as smoking and maternal dietary intake) may slow the rate of fetal development.[22-23] Despite this, children with cleft palate with or without cleft lip presented spontaneous recovery that starts at approximately 5 months of age. In addition, cleft lip children showed similar growth to healthy children.[38]

The main limitation of the present study was the analysis of the different types of orofacial clefts because the division of data into subgroups results in very small numbers, especially in cleft lip where the prevalence is lower. This may explain why only BMI showed differences statically significant among the different types of cleft studied. Additionally, unmeasured confounding factors cannot be excluded even though the model obtained presents an accuracy of 63.2%. This study has several strengths compared with the published literature: 1) both groups had similar socio-economic backgrounds; 2) controls (non-cleft group) were individually matched to each case regarding age and sex; 3) a geneticist participated in the diagnosis of the cleft group, avoiding including syndromic patients; and 4) data birth were recorded by medical professionals.

This study highlights the complexity in identifying the risk factors for having orofacial cleft, especially when trying to detect differences between phenotypes. In some countries all over the world, healthcare systems cannot afford treatment for cleft lip and palate. Identification of strategies to modify risk factors for non-syndromic OC (such as being overweight) is the first step toward primary prevention.[39] As strong evidence of some of the prevention factors is still lacking, the next reasonable step for research might be the observational studies that consider the interactions of several risk factors, namely maternal family history of diabetes.

5. Conclusions

In this homogenous population-based study, maternal body mass index and maternal age was found to affect the risk of having a cleft child. However, the association of these two factors does not increase the risk. In the children's initial data, the cleft group found a higher risk of having a lower birth weight but no relation was found regarding length and head circumference. This trial and further studies will allow the incorporation of the findings into preconception counseling health care programs for women of reproductive age.

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Chapter III

III

Health disparities in the treatment of cleft patients in Europe: a cross-sectional survey

1. Introduction

According to the World Health Organization, cleft care is still far from ideal due to service organization, inequalities of care, uncertainty around treatment and limited resources.[1] Over the past decades, several improvements in cleft lip and/or palate (CLP) treatment have been made with some high-income countries adopting national protocols and the centralization of services, which have some advantages: standardized data collection; a coordinated approach; better responsiveness to patient and family needs due to interdisciplinary teams; and continuous monitoring.[2-5]

In Europe, CLP affected 14.5 per 10 000 births between 2011 and 2018 according to the European Network for Epidemiological Surveillance of Congenital Anomalies report.[6] Cleft teams and treatment protocols for the management of patients with clefts vary considerably within and between European countries. In the late 1990s, Europe produced a set of recommendations for cleft care. This highlights that standardized procedures can allow for intercenter comparisons as well as the evaluation of treatment outcome in each individual center so as to aim for an improved in outcome should this be deemed necessary.[1]

In 2001, Shaw et al. reported that some countries in Europe did not yet have team care, and they also found that 194 out of 201 teams had different surgical protocols for only unilateral clefts.[2] Later in 2011, a United Nations International Children's Emergency Fund (UNICEF) survey in Bulgaria found that more than forty percent of parents of children with clefts were advised to leave their children in an orphanage.[7] The complexity of cleft treatment, which includes multiple surgeries and medical treatments, may explain this finding. It is estimated that the average cost for cleft children up to the age of 10 is eight times higher than for healthy children of the same age.[8] Access to healthcare varies widely between and within countries, which can influence the provision of care in this respect. Cleft research has several challenges such as the length of follow-up and the range of different outcomes, since clefts can lead to disturbances in many neighboring structures and their associated functions.[1] Therefore, international collaboration is required to obtain a sample with adequate power.[9-11]

The 2030 Agenda for Sustainable Development was launched in 2015 and defined 17 goals to be adopted by all United Nations Member States, recognizing the need to share knowledge, new technological advances and financial resources to achieve the sustainable development goals in all countries. Goal 3 is focused on ensuring healthy lives and promoting well-being for all at all ages, and reads: «Achieve universal health coverage, including financial risk protection, access to quality essential health-care services and access to safe, effective, quality and affordable essential medicines and vaccines for all».[12] In order to develop new sustainable strategies, fulfilling the aim of reducing inequalities in the 2030 Agenda, it is essential to recognize the current inefficiencies of cleft care. Despite the creation of the EUROCRAN project, which proposes to help international collaboration in Europe, the lack of evidence-based care in the field of orofacial clefting with only few randomized clinical trials being carried out is still a problem.[13] Furthermore, no recent efforts have been implemented to assess the current treatment approaches to CLP since the Eurocleft study in 2001. Therefore, this study aims to instigate a critical appraisal of cleft care in Europe, which will allow a better understanding of its main strengths and inefficiencies.

2. Materials and Methods

The study used a survey to investigate several areas of cleft care, namely, provider characteristics; patient profile; services offered; treatment protocols; complications, especially in orthodontic treatment. The Google documents platform was used to create an online survey with a unique URL, giving access to the survey at any time and anywhere in the world. The survey was available online from November 2020 until January 2021. For each question, participants place a tick on their answer from a list provided. Appendix 3.1 presents the survey questions and answers.

A preliminary register was compiled through association membership lists with a known email address, namely Orphanet, European Cleft Organisations, the European Orthodontic Society and the European Federation of Orthodontic Specialist Associations. Subsequently, an email was sent to the mailing list established. Reminder emails were sent at 2, 4 and 8 weeks to nonresponders. After that, the survey was closed to new responses. The data was automatically stored using a "cloud" database. The unique study ID ensured confidentially of all data. An automated method then generated the numerical values, allowing data to be imported into the Statistical Package for the Social Sciences, version 24.0 for Windows (SPSS Inc., Chicago, Illinois, USA). Duplicate entries were rejected. Incomplete questionnaires were excluded. Descriptive statistics (frequency and, when applicable, means and standard deviations) were calculated for each question. The association between categorical variables was performed using Fisher's exact test. The significance level was set at $p < 0.05$.

3. Results

Survey responses and covered countries

Seventy-nine out of 214 individuals on the mailing list responded to the survey (37%), of which 10 were excluded due to an incomplete questionnaire. Sixteen participants decided not to participate because they do not treat CLP patients. Overall, 69 individuals completed the entirety of the survey (56.5% female and 43.5% male), with a response rate of 36.7% (69 out of 188). The responding participants included 52 (75.4%) orthodontists, 9 (13%) maxillofacial surgeons, 5 (7.2%) plastic surgeons, 2 (2.9%) pediatric surgeons and one (1.4%) speech and language therapist.

This survey includes a sample from 23 European countries of which Switzerland, the United Kingdom and Italy had the highest response rate (Figure 3.1).

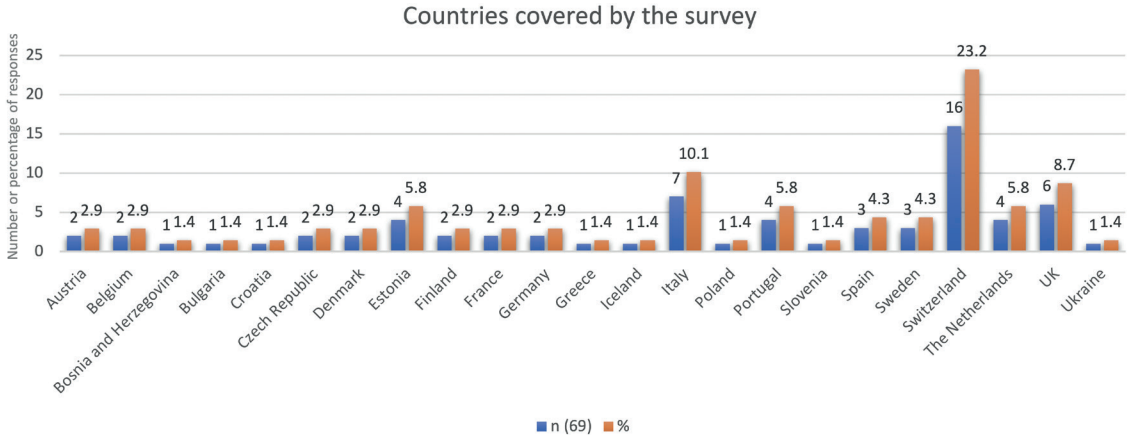

Figure 3.1. List of countries covered by the survey

Providers' characteristics / practice management

The average number of years of professional experience was 21.6 (SD=8.9). The respective centers/offices collaborated in the treatment of CLP patients for an average of 31.1 years. An average of 73.9% of respondents work in a hospital and/or university environment.

Centralization of services (compared to local services) was the preferred reference system, with some countries (Bulgaria, Croatia, Czech Republic, Denmark, Finland, Slovenia, Sweden, The Netherlands) reporting that it was the only reference system used (Table 3.1). It was also reported that the majority of patients lived less than 51 kilometers away (46.4%). Involvement of several medical specialties was reported, with orthodontics (98.6%) and maxillofacial surgery (81.2%) being the most frequently mentioned.

The majority of countries have a CLP association for patients and professionals (53.6%) (Table 3.1). However, five countries reported only parents' associations (Czech Republic, Estonia, Finland, Iceland, and Slovenia). Of all 69 participants, 36 reported being a member of professional associations (52.2%).

Table 3.1. Survey results on practice management

Question	Response
Please select where your center/office is integrated:	
Hospital environment;	17.4%
University environment;	24.6%
Private practice;	26.1%
University hospital environment.	31.9%
What is the preferred reference system for CLP patients in your country?	
Centralized services;	43.5%
Local services;	14.5%
Both;	36.2%
Not sure/None of the previous answers.	5.8%
How far from your center/office are the majority of your CLP patients?	
0-50 km;	46.4%
51-100 km;	30.4%
101-150 km;	13.0%
> 150 km.	10.1%
Select the medical specialties involved in CLP treatment in your department/office?	
Otorhinolaryngology;	65.2%
Maxillofacial surgery;	81.2%
Plastic surgery;	50.7%
Neurosurgery;	11.6%
Orthodontics;	98.6%
Pediatric surgery;	30.4%
Phoniatrics/Speech Therapy;	72.5%
Dentistry;	71.0%
Geneticist;	47.8%
Child and adolescent psychiatry;	42.0%
Clinical nurse specialist.	42.0%
Are there national CLP associations in your country?	
Yes, only for professionals;	13.0%
Yes, only for parents;	17.4%
Yes, for both;	53.6%
No or not sure.	15.9%

Characterization of patients

Most respondents reported that the majority of patients undergoing treatment are under 13 years of age (Figure 3.2). Hospital-based practices also had a statistically significant association with three age groups: 4-6 years (Fisher's exact test, p = 0.01); 18-35 years (Fisher's exact test, p = 0.04); over 35 years (Fisher's exact test, p = 0.01). The remaining age groups do not differ in relation to the place of the delivery of services. The most prevalent diagnosis was unilateral cleft lip and palate (Figure 3.2), followed by isolated cleft palate, bilateral cleft lip and palate, and finally isolated cleft lip.

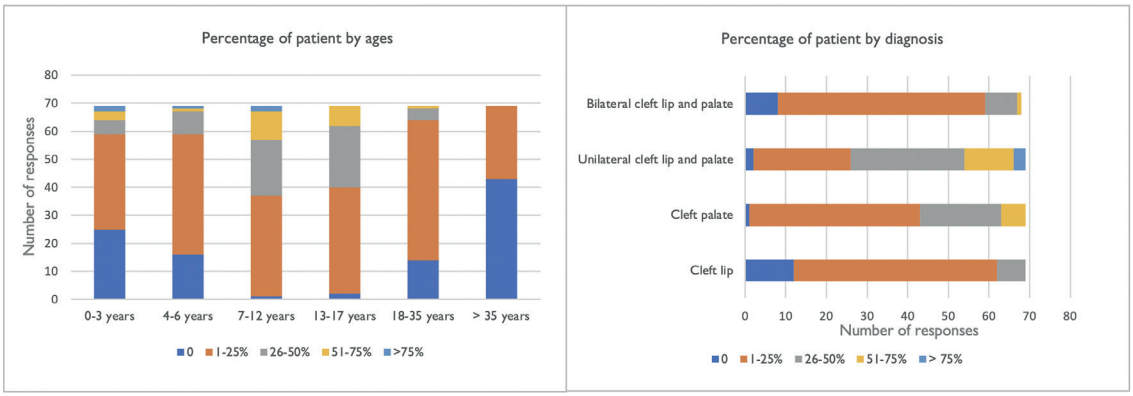

Figure 3.2. Distribution of patient by ages and diagnosis

The largest percentage of patients were seen in a university hospital environment (Fisher's exact test, p < 0.001) (Table 3.2). An increase in the volume of patients was found, particularly in the university and/or hospital environment, when comparing current data with data from the last 3 years. Private practice did not register significant changes, evaluating 100 patients or less in most responses.

Table 3.2. Survey results on distribution of CLP patients by office environment

0-100		How many CLP patients in the last 3 years			
		101-200	201-300	> 300	
Office is in	Hospital	3	1	3	5
	University	11	3	2	1
	Private practice	17	0	1	0
	University hospital	7	4	1	10

0-100		How many CLP patients currently			
		101-200	201-300	> 300	
Office is in	Hospital	5	0	0	7
	University	12	2	0	3
	Private practice	16	1	1	0
	University hospital	4	3	5	10

Treatment protocols

Of the 69 responses, this survey showed 50 different treatment protocols. The most frequently reported protocol sequences were: pre-surgical orthopedics; lip closure; soft palate closure; hard palate closure; and dentofacial orthopedics. In most reported sequences, three procedures were consistently referred to: lip closure, soft palate closure and hard palate closure (Table 3.3.). In contrast, the least reported procedure was gingivoperiosteoplasty (8 out of 50 possible sequences). On average, the centers performed 4.58 procedures in cleft treatment and only one center reported performing all the procedures listed.

Table 3.3. Sequence of treatment protocol. The numbers in the columns represent the responses given per procedure in chronological order.

	Sequence of treatment protocol											
	1	2	3	4	5	6	7	8	9	10	11	12
Pre-surgical orthopaedics	24	3	1	1	2	0	0	1	0	2	0	0
Lip adhesion	11	9	1	0	0	0	0	0	0	0	0	0
Lip closure	14	26	10	1	0	0	0	0	1	0	0	0
Gingivoperiosteoplasty	1	1	6	1	3	3	0	0	1	2	0	1
Soft palate closure	5	12	18	15	1	0	0	0	0	0	0	0
Hard palate closure	1	7	14	13	8	3	2	0	0	0	0	0
Orthophony	0	1	3	5	7	4	3	0	0	0	0	0
Velopharyngoplasty	1	0	0	7	4	8	3	6	0	0	1	0
Alveolar bone grafting	0	1	2	9	8	11	10	4	4	0	0	0
Dentofacial orthopaedics	4	1	0	2	12	11	4	5	2	3	0	0
Orthognathic surgery	0	1	1	1	5	7	16	6	7	4	5	1
Lip/Nose Revision	0	0	0	0	3	5	7	16	3	6	5	6

Regarding orthodontic treatment, the majority of orthodontists spend approximately 76 to 100 percent of their time treating patients with CLP (Table 3.4). There is a statistically significant association (Fisher's exact test, $p < 0.001$) between the time spent and the location of the office, with orthodontists in university hospital environments tending to spend more time on CLP treatment. The most used tool for orthodontic diagnosis was cone beam computed tomography (38.9%), but no statistically significant association (Fisher's exact test, $p = 0.05$) was found between diagnostic tools and medical specialties. Overall, 70.9% of responding participants performed pre-surgical orthopedics, and unilateral and bilateral CLP were the phenotypes where it was most performed. The presence of reported complications from orthodontic or surgical treatment was relatively low (0-25%), with postoperative fistula being the most frequent complication following surgical procedures (Table 3.4).

Table 3.4. Survey results on orthodontic care

Question	Response
What percentage of the time is an orthodontist present at your multidisciplinary clinic?	
0-25%;	21.7%
26-50%;	13.0%
51-75%;	8.7%
76-100%.	56.5%
What is the most used tool for orthodontic diagnosis and treatment planning in your center/office for CLP patients?	
Panoramic and cephalometric radiograph;	31.3%
Cone beam computed tomography;	38.9%
Computed tomography;	24.3%
Other.	5.6%
How often do you perform pre-surgical orthopedics on CLP patients in your clinical practice?	
Always;	18.8%
Often;	30.4%
Sometimes;	21.7%
Never.	29.0%
In which cleft phenotypes do you perform pre-surgical orthopedics?	
Cleft lip;	17.4%
Cleft palate;	29.0%
Unilateral cleft lip and palate;	56.5%
Bilateral cleft lip and palate;	62.3%
Pierre Robin sequence;	36.2%
Other.	13.0%
What percentage of CLP patients have complications during orthodontic or surgical treatment?	
0-25%;	90%
26-50%;	9%
51-75%;	1%
76-100%.	0%
What is the most frequent complication of CLP patients after surgical procedures:	
Postoperative fistula;	43%
Postoperative infection;	10%
Postoperative airway complication;	9%
Revision;	13%
Other.	32%

4. Discussion

This survey attempts to understand the current situation with regard to cleft care in Europe since the Eurocleft study in 2000. In particular, years of professional and center experience, reference system, specialties involved in cleft treatment, presence of national associations, treatment protocols and some concerns about orthodontic treatment were analyzed in this study.

A sample of 23 European countries were included in this study. Comparing this sample with the Eurocleft study, it was possible to ascertain: 1) inclusion of two countries: Bosnia and Herzegovina and Croatia; 2) no responses were obtained from the following countries: Slovakia, Romania, Norway, Latvia, Ireland and Hungary.[2] Despite these differences, we feel that these studies are similar enough to allow us to compare the main strengths and inefficiencies in cleft care found. Furthermore, the high average of professional/center experience found in this survey (21.6/31.1 years) permits a reflection on the current practice of professionals who treat patients with CLP.

Regarding the reference system, few improvements were found with some countries adopting: centralized services – Finland, Bulgaria and Spain; or a combination of localized and centralized services – Greece, Italy, Ukraine, Portugal, France and Germany. Ness et al. suggested the relevance of having centralized multidisciplinary services in all countries, because small centers with low samples have a great difficulty in proving the quality of their outcomes.[14] To date, few adequately powered randomized clinical trials exist, and centralized services with or without a state-funded health system would allow for easier and more accurate clinical research.

Patients with CLP spend many years undergoing corrective procedures which require a multidisciplinary team. The majority of responders reported the involvement of several medical specialties in cleft treatment and this holistic approach is in line with American Cleft Palate-Craniofacial Association (ACPA) minimal standards for the cleft palate team (orthodontist, surgeon, and speech-language pathologist).[15] Although the three most reported specialties are similar to the Eurocleft study (otorhinolaryngology, maxillofacial surgery and plastic surgery), others specialties have been referred to as being important in cleft treatment, namely orthodontics, dentistry and phoniatrics/speech therapy.

National associations showed an improvement with the creation of associations for parents and professionals in some countries, for example in Portugal. Unfortunately based on results of this survey, countries like Bosnia and Herzegovina, Greece, Poland and Ukraine still have no organized association. The creation of associations in these countries may help to spread accurate information to patients, families/caregivers, and professionals, and to identify essential characteristics of quality for team composition.

In general, the majority of patients with CLP are followed up in a university hospital environment. This contrasts with the 2018 study by Khavanin et al., who reported private practice (58.6%) as the current model of CLP orthodontic care provision in the United States of America (USA). The authors also reported that, in the responders' opinion, the ideal model for orthodontic care is a combination of university/hospital and private practice.[16] In Europe, CLP treatment is often supported by National Health Service while in the USA only 2% of community orthodontists accept Medicaid, which can explain the differences in the model of care. According to Khavanin et al. study, financial and insurance concerns create difficulties in the delivery of cleft orthodontic care in USA.[16]

Based on the present results, hospital-based practices follow more patients in three specific age groups (4-6 years; 18-35 years; over 35 years). This may be explained because these age groups are usually associated with surgical procedures, namely soft and hard palate closure at 4-6 years, orthognathic surgery at 18-35 years, and lip/nose revision in patients over 35 years.

Since the previous Eurocleft intercenter study, standardization of the cleft care protocol had been suggested as desirable. In the present survey, we found less heterogeneity of protocols (50 protocols for 69 responders), with the percentage of different protocols still being high, i.e. 75% compared to 96% in 2000.[2] These results are disappointing, considering the 21-year interval between the two studies. Since timing and treatment sequences vary, the aggregation of data is complex, which explains the lack of supporting evidence in this field.

The majority of respondents (98.6%) reported that an orthodontist was involved in CLP treatment, with 56.5% of them spending 76-100% of their time treating patients with CLP. The ACPA study reported distinct results with only 32.8% of their orthodontists dedicating a majority of their time to cleft care.[16] These contradictory data may be associated with the place of service delivery, since in Europe orthodontists are integrated into a university hospital environment while in USA the majority of orthodontists are associated with private practice. Additionally, this may also explain why Europe seems to offer presurgical infant orthopedics more often than in the USA (70.9% vs 48.6%) since orthodontists who are less experienced in managing patients with CLP are less likely to provide these treatments.[16]

The most preferred diagnostic tool prior to orthodontic treatment was cone beam computed tomography (CBCT). Despite the Eurocleft study using lateral cephalograms, CBCT has become a diagnostic imaging tool in patients with CLP since it had a higher definition than conventional two-dimensional methods and lower radiation exposure compared with computed tomography.[17-18] The American Academy of Oral and Maxillofacial Radiology defined clinical recommendations regarding the use of CBCT and this included imaging in the context of CLP care, since these patients have medical conditions that required proper 3D analysis for accurate diagnosis.[19]

As far as complications during orthodontic and surgical treatment are concerned, this study shows a low reported incidence of these complications, which is in accordance with a recent systematic review that showed an overall perioperative complication rate of 12.6%, with postoperative fistula being the most frequent complications.[20]

The main limitation of most surveys is the low response rate, which was also relatively low in the present study. Even though we hoped the web-based approach would motivate more responses, the lack of compliance can be partly explained by the size of the questionnaire and the presence of few specialized centers for cleft care in Europe. However, the rate of responders is similar to the last Eurocleft study (40%), which allows a comparison of the results of both studies and highlights the current practice in cleft care among European countries.

5. Conclusion

This survey provides updated information on the current organization of cleft care within Europe. Based on the results, the achievement of a consensus with regard to cleft care across Europe remains suboptimal and challenging. Therefore, there is a need for better coordination between clinicians and national and international regulatory bodies and centers. Studies focusing on how the providers' characteristics and practice management issues influence the outcome of cleft treatment are still required.

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IV

Chapter IV

Current cleft orthodontic care in Europe: a cross-sectional survey

1. Introduction

Cleft lip and palate (CLP) is one of the most frequent craniofacial malformations, affecting about 14.5:10 000 births between 2011 and 2018 in 26 European countries.[1] Management of patients with CLP requires an interdisciplinary team of specialists to achieve normal speech, hearing and occlusion with a normal facial appearance and psychological well-being.[2]

Several organizations have attempted to provide recommendations to reduce the burden in patients with CLP, notably the World Health Organization and the American Cleft Palate-Craniofacial Association (ACPA).[3-5] In 2015 the latter association defined minimum standards for the inclusion in cleft palate teams, namely the fulfilment of the eight basic criteria and 30 of the 35 additional criteria defined by the ACPA. The following criteria are relevant: the team must include an active orthodontist, surgeon and speech-language pathologist; the orthodontist must have treated at least ten patients with cleft lip and/or palate in the prior year to the team's application; and an orthodontist capable of providing orthodontic treatment as a part of orthognathic treatment.[6]

Despite improvements in both aesthetics and function, the surgical protocols used can lead to abnormal craniofacial development, associated with the formation of post-operative scar tissue.[7] Moreover, dental anomalies are significantly more frequent in patients with CLP than in the general population, with studies reporting that about 94% of patients with CLP present at least one dental anomaly.[8] The orthodontist has an important role in the interdisciplinary team, since some characteristics of patients with CLP may lead to the development of malocclusions (eg anterior or posterior crossbites, open bite, skeletal Class III and crowding).[9-10] However, the majority of studies regarding orthodontic treatment in patients with CLP present differences in the treatment protocols, measurement of outcomes (eg cephalometric analysis), characteristics of retrieved data, and differences between control groups.[11-12]

The Eurocleft network, involving 30 countries and 201 centres, highlighted the differences in cleft teams and treatment protocols in European countries. In 2001, some of these countries did not yet have an interdisciplinary team for cleft care.[13] Furthermore, significant variations were reported

between centres, namely a mean of 3.5 to 6 surgical procedures, a length of orthodontic treatment of 3.3-8.5 years, with a range of 49-94 orthodontic visits.[14] After these disappointing findings, the EUROCRAN project emerged in order to promote international collaboration and to study genetic and environmental risk factors for CLP, as well as prevention measures and treatment approaches.[15] More recently, a European cleft and craniofacial initiative for equality in care network has also been created to promote the sharing of research methods and knowledge of treatments.[16]

In the 18 years since the Eurocleft studies, healthcare systems all over Europe have undergone a significant improvement but several discrepancies between provider characteristics, treatment protocol or appliances offered and financial support still persist.[5,17] These data might reveal that the recommendations of the aforementioned European projects were not implemented consistently. Standardized care will help to distribute available resources in a sustainable fashion and reduce the overall burden and cost of treatment. Thus, the aim of this study was to instigate a critical appraisal of orthodontic treatment in patients with CLP including provider characteristics, orthodontic appliances, services offered, orthodontic complications and cost analysis.

2. Materials and Methods

A cross-sectional 22-question survey was created to investigate several factors related to the provision of orthodontic cleft care, namely characteristics of care providers, size of treatment centres, orthodontics appliances offered, orthodontic complications and financial support. For each question, participants had to tick their answers from a list provided (Appendix 4.I).

The survey was developed using the Google documents platform with a unique URL, which allowed it to be accessed anywhere and at any time in the world.

An email was sent with the survey link through the collection of contacts obtained through cleft and orthodontic association membership lists, namely the European Federation of Orthodontic Specialist Associations, the European Orthodontic Society, the European Cleft Organisation and Orphanet. Unresponsive contacts were reminded by email at 2, 4 and 8 weeks after initially sending the link. The online survey was accessible from November 2020 until January 2021. The data were automatically stored with a unique study ID, ensuring the confidentiality of all data. An automated method generated the numeric data into an Excel spreadsheet (Microsoft Corporation, Redmond, WA). Double entries and answers with disparities were manually rejected in order to create a final data set.

The final data were extracted to the Statistical Package for the Social Sciences, version 24.0 for Windows (SPSS Inc., Chicago, Illinois, USA). Descriptive statistics were calculated for each question. Fisher's exact test was used to assess the association between categorical variables. The significance level was sent at $p<0.05$.

3. Results

A total of 79 responses were obtained from 214 individuals contacted (response rate 36.9%). Of these, ten responses were excluded due to the participants not having complete the entirety of the survey. The final sample thus consisted of 69 responses from 23 European countries (Figure 4.1).

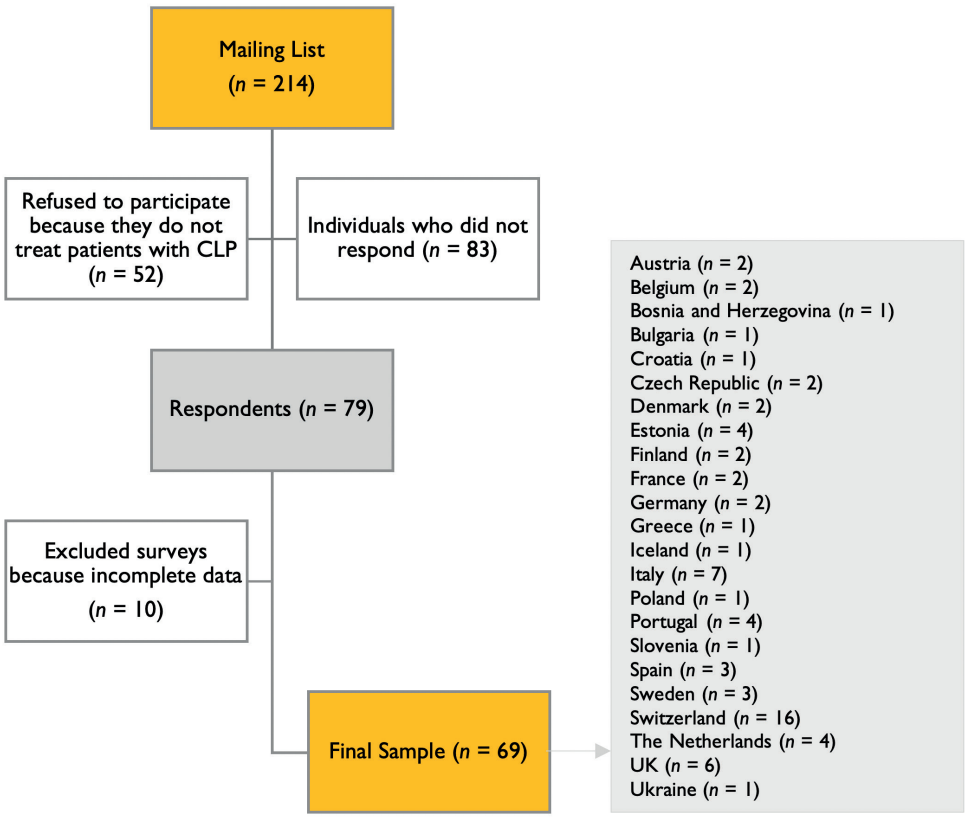

Figure 4.1. Flow chart of the acquisition of the final sample

The data from the surveys revealed that in most cleft teams, orthodontists are present in the multidisciplinary team meetings more than 75% of the time, but the time dedicated to CLP care, by these orthodontists, is less than 26% (Table 4.1). No statistically significant association (Fisher's exact test, $p = 0.919$) was found between the percentage of time that orthodontists are present in the multidisciplinary team meetings, and the environment in which the centre/office is integrated (private practice or hospital and/or university environment).

The majority of the respondents (70.9%) reported offering pre-surgical orthopaedics, with bilateral CLP being the most common phenotype in which these appliances were used (Table 4.1).

Table 4.1. Survey results on orthodontic care

Question	Response
Please select where your centre/office is integrated:	
Hospital environment;	17.4%
University environment;	24.6%
Private practice;	26.1%
University hospital environment.	31.9%
What percentage of the time is an orthodontist present at your multidisciplinary clinic?	
0-25%;	21.7%
26-50%;	13.0%
51-75%;	8.7%
76-100%.	56.5%
What percentage of your orthodontist's practice is devoted to the care of CLP patients?	
0-25%;	57%
26-50%;	16%
51-75%;	17%
76-100%.	10%
How often do you perform pre-surgical orthopaedics on CLP patients in your clinical practice?	
Always;	18.8%
Often;	30.4%
Sometimes;	21.7%
Never.	29.0%
In which cleft phenotypes do you perform pre-surgical orthopaedics?	
Cleft lip;	17.4%
Cleft palate;	29.0%
Unilateral cleft lip and palate;	56.5%
Bilateral cleft lip and palate;	62.3%
Pierre Robin sequence;	36.2%
Other.	13.0%
In which cleft phenotypes do you perform nasoalveolar moulding?	
For patients with unilateral cleft lip and palate;	21.7%
For patients with bilateral cleft lip and palate;	21.7%
For all complete cleft lip and palate cases;	18.8%
Never.	55.1%
What is the usual maxillary expansion protocol that your team uses in CLP patients?	
Rapid maxillary expansion;	44.9%
Semi-rapid maxillary expansion;	23.2%
Slow maxillary expansion;	39.1%
Alt-RAMEC.	8.7%

Question	Response
What appliance do you normally use for maxillary expansion?	
Removable expansion plate;	23.2%
Quad helix;	53.6%
Hyrax/Haas;	55.1%
W-Arch;	8.7%
NiTi expander;	2.9%
Spring Jet;	14.5%
Other tooth-borne appliance;	2.9%
Bonded expansion plate;	15.9%
Tooth-tissue-borne appliance;	23.2%
Bone-borne appliance.	53.6%
In CLP cases with a missing maxillary lateral incisor, which treatment approach do you prefer?	
Substitute the maxillary canines as the lateral incisor;	27.5%
Prosthetic replacement;	4.3%
No preference. The treatment approach depends on the clinical case;	68.1%
Other.	27.5%
After the alveolar graft, how long do you wait until you start moving teeth to the newly grafted area?	
1 month;	7.2%
2 months;	14.5%
3 months;	34.8%
4 months;	2.9%
5 months;	1.4%
6 months;	34.8%
Other.	4.3%
What alternative treatments do you use instead of secondary bone grafts?	
Distraction osteogenesis;	18.8%
Tongue flap and secondary bone graft;	2.9%
BMP-2;	5.8%
None;	58.0%
Other.	17.4%
Do you make other appliances (speech bulbs, etc.) for CLP patients?	
Yes;	27.5%
No.	72.5%

Maxillary expansion is commonly used in the treatment protocol in patients with CLP. Of all maxillary expansion protocols, rapid maxillary expansion was the preferred one (44.9%), but no statistically significant differences were found (Fischer's exact test, $p = 0.886$) between different types of expansion. Furthermore, no association was found between the percentage of time a practitioner spent treating patients with CLP and the protocol or type of orthodontic appliance used for maxillary expansion (Table 4.2). Various types of orthodontic expansion appliances were used.

Table 4.2. Correlation between CLP practice time and the protocol or type of orthodontic appliance

		Fisher's exact test
Protocol expansion	Rapid maxillary expansion	p = 0.361
	Semi-rapid maxillary expansion	p = 0.212
	Slow maxillary expansion	p = 0.406
	Alt-RAMEC	p = 0.244
Expansion appliances	Removable expansion plate	p = 0.244
	Quad helix	p = 0.313
	Hyrax	p = 0.105
	Haas	p = 1.000
	NiTi expander	p = 0.684
	Other tooth-borne appliance	p = 0.413
	Tooth-tissue-borne appliance	p = 1.000
	Bone-borne appliance	p = 0.133

Distraction osteogenesis was the most reported alternative treatment to secondary alveolar bone grafts (18.8%) (Table 4.1). Additionally, it was found that those in private practice are less likely to perform alternative treatments (Fischer's exact test, p = 0.001). Regarding the timing of initiating tooth movement into the newly grafted area, the results were not homogeneous, with 3- and 6-months post-grafting being the most reported (Table 4.1).

The provision of orthodontic services was similar in the four types of practice locations (private practice, hospital, university, hospital and university environment): dentofacial orthopaedics (Fisher's exact test, p = 0.527); orthodontic treatment only (Fisher's exact test, p = 0.587); orthodontic surgical treatment (Fisher's exact test, p = 0.110). Figure 4.2 describes the distribution of the patient population by the type of orthodontic treatment carried out.

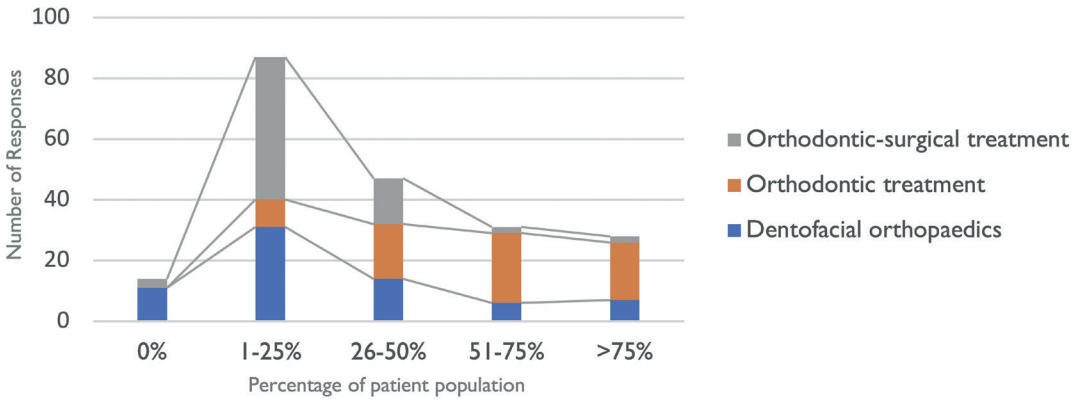

Figure 4.2. Distribution of the patient population by the type of orthodontic treatment

Orthodontic procedures were reported to have few complications, in which compromised oral hygiene (77%) was the most reported (Table 4.3). Regarding pre-surgical orthopaedics, complications were mainly skin sores from the tape (28%).

Table 4.3. Complications of orthodontic procedures

Question	Response
What percentage of CLP patients have complications during orthodontic or surgical treatment?	
0-25%;	90%
26-50%;	9%
51-75%;	1%
76-100%.	0%
What is the most frequent complication of pre-surgical orthopaedics?	
Interference with growth;	12%
Delaying surgery;	9%
Occlusion of the airway;	1%
Skin sores from the tape;	28%
Ulceration under a plate;	16%
Risk of infection under a plate;	3%
Feeding problems;	6%
Other.	41%
What is the most frequent complication of orthodontics treatment?	
Expansion relapse;	52%
Ulceration;	9%
Compromised oral hygiene;	77%
Phonetic problems;	13%
Mastication problems;	7%
Dysphagia;	0%
Other.	4%

Regarding cost analysis, it was found that the respective National Health Services support the majority of cleft orthodontic care (67%) and most patients reported to their medical team that they were satisfied with the services provided (74%) (Table 4.4). More than half of respondents (61%) reported their patients having more than six appointments per year and the majority living less than 101 kilometres away (76.8%).

Table 4.4. Cost analysis

Question	Response
How far from your centre/office are the majority of your CLP patients?	
0-50 km;	46.4%
51-100 km;	30.4%
101-150 km;	13.0%
> 150 km.	10.1%
How many appointments do CLP patients have at your centre/office each year?	
0-3;	23%
4-6;	16%
7-12;	49%
>12.	12%
How do CLP patients typically pay for orthodontic services?	
National Health Service;	67%
Out-of-pocket expenses (self);	10%
Insurances;	7%
National Health Service and self-paid;	12%
Insurances and out-of-pocket expenses (self).	4%
Are your patients satisfied with their current level of access to orthodontic services?	
Dissatisfied;	1%
Neutral;	10%
Satisfied;	74%
Not sure.	14%

4. Discussion

This study attempted to summarize the current provision of CLP orthodontic treatment in European countries. It also provides a critical assessment of the implementation of the recommendations defined by the Eurocleft studies and European projects.

Several descriptions of orthodontists' role in the treatment of patients with CLP have been published but significant variations have been reported concerning the availability of the orthodontist and the type of services offered.[5,14] This survey found that few orthodontists (10%) dedicated more than 75% of their time to CLP treatment. Two reasons may account for this. State-supported institutions (university and/or hospital) may have more difficulties in hiring full-time orthodontists due to financial restrictions. Additionally, 26.1% of respondents practise in a private environment, which may also contribute to the underestimation of this percentage. The present survey reported an improvement since the Eurocleft study, where the orthodontist was not recognized as one of the main specialties involved in cleft surgery, but presurgical orthopaedics was used by 65% of teams.[13] More recently, in 2014, Scott et al. investigated the current provision of cleft services in the United Kingdom, and found that primary cleft surgery and orthodontics were the only medical specialities represented in all teams.[17] Additionally, Khavanin et al.'s study, published in 2019, showed that the majority of orthodontists integrated in cleft teams in the United States of America (USA) were private practice volunteers (48.6%), and only 17.1% devote a majority of their practice (75% to 100%) to cleft care.[5] Although there is a discrepancy in the location of provision between Europe and the USA, the time devoted to CLP treatment is similar.

Regarding orthodontic appliances, this study was planned to examine the current provision of pre-surgical orthopaedics, maxillary expansion and other appliances (eg speech bulbs). Despite the potential stated advantages of presurgical orthopaedics (eg cleft reduction, which promotes less lip tension and benefits wound healing postoperatively), only half of the respondents performed nasoalveolar moulding, which may be associated with the lack of evidence on long-term outcomes.[18-20] In contrast, the role of maxillary expansion is already recognized as part of the treatment protocol

for patients with CLP with some goals such as the correction of the transverse discrepancy, the establishment of the maxillary arch form and the availability of space for secondary bone grafts.[21] It was found that respondents preferred the rapid maxillary expansion protocol. Previous studies suggest slow instead of rapid maxillary expansion based on the following assumptions: a) patients with CLP have a disturbed, irregular or absent palatal suture system, which increases the response to orthopaedic forces; b) rapid maxillary expansion had some disadvantages such as requiring patient cooperation in activation, the creation of an open bite, micro trauma of the temporomandibular joint, root resorption, tissue impingement and pain; c) slow maxillary expansion produces less tissue resistance around the circummaxillary structures, which improves bone formation in the intermaxillary suture.[22] However, recent studies show that both expansion protocols have similar effects.[23] The choice for the design of the expander may depend on factors such as practitioner preference or the localization of the constricted region of the maxilla. Both hyrax and quad-helix expanders can be used when anterior and posterior maxillary expansion is needed, which may explain why these appliances were the most mentioned by the respondents.[24] Despite the potential benefit of a speech bulb in reducing hypernasality when velopharyngeal insufficiency is present, only a minority of responders (27.5%) offer this appliance.[25] This may be due to the improvement of surgical protocols and the reduction in the necessity and indication of these appliances.

After the secondary alveolar bone graft, orthodontic treatment should be restarted in order to move the teeth adjacent to the cleft area into the newly grafted bone, thus improving the consolidation of the alveolar bone and the height of the crest. The timing to move teeth into the grafted bone is not well established in the literature; the most reported timing in the present survey was three to six months post-grafting.[26]

This survey shows that the offer of common orthodontic services is similar in the various locations of provision (private practice, hospital, university, hospital and university environment), but the offer of less common treatment approaches may vary (less likely in private practice). These findings could be partially explained for several reasons such as: university or hospital centre have several specialities on-site; contact with high volumes of patients allows more training in cleft management; and university or hospital centres enjoy considerably higher rates of treatments covered by the National Health Service than privately, allowing patients to access more expensive treatments, namely distraction osteogenesis.

The orthodontic burden of care usually is higher in patients with CLP than in patients without this condition. Few complications regarding orthodontic treatment were reported (90% of respondents reported less than 26% complications), and the most reported complication was compromised oral hygiene. Patients with CLP may have more difficulty in performing adequate oral hygiene practices due to factors related to the higher incidence of supernumerary teeth, the presence of malocclusions, the presence of viscous nasal fluid that may accelerate the adherence of plaque, and the healing tissue after surgical procedures that may make oral hygiene more challenging.[27] The duration of treatment also contributes to orthodontic burden because patients with CLP may require more appointments (44 vs 18 appointments in healthy patients) due to the complexity of treatment.[28-29]

The majority of respondents mentioned that the National Health Service supports cleft orthodontic care (67%). A distinct finding was obtained by Khavanin et al., showing that only a minority of orthodontic treatment was paid for by state-funded programme in the USA.[5] This reveals the efforts of many countries within Europe to provide equal access to orthodontic treatment, respecting the third goal of the 2030 Agenda (ensure healthy lives and promote well-being for all at all ages). Moreover, most respondents reported that patients were satisfied with the orthodontic treatment offered (74%), which may be due to the variety of services available as well as the proximity of the centre/office of provision (most patients live less than 101 kilometres – 76.8%).

A limitation of the present study is that the data may be biased because respondents provided their own subjective interpretations and perceptions. However, this survey represented a variety of practice settings which may minimize this bias. The second limitation of this survey is the low response rate (37%). However, this rate is similar to other studies, namely the Eurocleft study (response rate = 40%) and the ACPA study about orthodontic care (response rate = 49.1%). Despite the fact that these findings have to be interpreted with caution, they might be considered to be reflective of the current orthodontic practice in patients with CLP in Europe.

A significant improvement in orthodontic treatment provision was observed in Europe but evidence in cleft treatment is still sparse since the results of current studies are heterogeneous due to several factors such as heterogeneous samples, different treatment protocols and inadequate follow-up. Mossey et al. suggested the establishment of prospective registries to accelerate collaborative monitoring and critical appraisal (phase I trials).[30] This would allow practice guidelines based on evidence to be established, improving surgical and orthodontic outcomes and reducing the burden of care, thus resulting in a reduced overall cost to the patient and society.

5. Conclusion

Europe has undergone an apparent improvement in orthodontic care over the past 18 years but several discrepancies still exist, namely the treatment timing protocol and appliances offered. Further research should be focused on the role of the orthodontic services in the outcome of cleft treatment.

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Chapter V

V

A comparative study of oral health-related quality of life among cleft patients and their families during orthodontic treatment

1. Introduction

Treatment of patients with cleft lip and palate (CLP) is considered one of the most challenging, since CLP leads to several difficulties such as feeding, hearing, speaking, breathing and dentofacial development.[1] The presence of dental anomalies and abnormal craniofacial development can lead to the development of malocclusion such as crossbite (anterior or posterior), open bite, skeletal Class III and crowding.[2] Dental anomalies are significantly more frequent in CLP patients than general population, leading to a long-term impact on patient's facial anatomy and self-esteem.[3]

Over the past few years, it has been recognized that CLP condition may affect oral health-related quality of life (OHRQoL).[4] In an earlier study, Montes et al. showed that unilateral CLP patients reported more orofacial dysfunctions and negative impacts on social well-being than controls.[5] Age, sex and cleft phenotype influence the OHRQoL score. Typically, older ages and female had a higher impact on OHRQoL in CLP patients, but these results are still controversial in the literature.[6] Moreover, CLP patients can experience lower OHRQoL in physical pain and psychological discomfort domains despite the comprehensive treatment received.[7] The rehabilitation of CLP patients requires an interdisciplinary team, in which the orthodontist plays a fundamental role from birth to adulthood.

As for the orthodontic treatment, it has been shown in the literature that it can influence OHRQoL: 1) malocclusion in aesthetic zone was associated with worst OHRQoL score[8]; 2) patients with worst OHRQoL are more likely to seek further treatment than patients with high OHRQoL[9]; 3) children with low psychological well-being can benefit from orthodontic treatment[10]; 4) adverse side effects of orthodontic treatment may decrease OHRQoL, such as pain and anxiety[11]; 5) patients who had completed orthodontic treatment had a better OHRQoL than those under treatment or who never had treatment[12]; 6) during orthodontic treatment, OHRQoL is worse in oral symptoms and functional limitations domains but better in emotional well-being.[13]

A recent review of outcomes related to orthodontic treatment in CLP patients found that quality of life and health resource utilization are the outcomes with the least representativeness in the literature.[6] To date, most studies in this field found that surgical patients had a significantly greater

reduction in OHRQoL score than patients with severe malocclusions or cleft patients.[14] Barros et al. compared Class III malocclusion with unilateral CLP and suggested that OHRQoL appears to be more affected by the etiology of Class III than by surgical or orthodontic treatment.[15] Despite this, the impact of orthodontic treatment on CLP patients without considering surgical approaches remains ambiguous.

Additionally, the continuous demands imposed on the caregiver during CLP patient's rehabilitation influence caregivers' well-being. The impact on OHRQoL appears to be similar between CLP patients and their caregivers.[16] Beluci et al. reported that caregivers with greater burdens have a worse perception of the quality of life. They also described that the perception of rehabilitation and the coping capacity of CLP children are influenced by the expectations, attitudes and support of their parents.[17] Difference on OHRQoL scores by children and their parents were reported with parents underestimate the "oral symptoms" but overestimate "functional well-being" of having a cleft.[18] Psychologic events, such as stigma and discrimination among peers due to CLP condition, may also affect the quality of life of patients and their families.[19] Moreover, Raghavan et al. found that parents rated the malocclusion more critically than CLP patients.[20] The effect of orthodontic treatment in patients without craniofacial disharmony is accepted to be highly beneficial on the families.[21] Therefore, the rehabilitation of CLP patients must also focus on family's needs approach. As far we are aware, no study had evaluated OHRQoL of parents regarding CLP children undergoing orthodontic treatment.

Knowledge on OHRQoL improves treatment quality, since it helps to identify the functional, emotional and social impacts, contributing to the planning of health care and clinical interventions.[4] This is even more important in CLP patients since they have a lengthy orthodontic treatment. Despite the increase in the number of studies involving quality of life and orthodontic treatment, there is little evidence in the evaluation of the impact of orthodontic treatment on CLP patients and their families. The aim of this study was to assess whether orthodontic treatment affected the levels of OHRQoL in CLP patients and their families. The following hypothesis are proposed:

1. There are differences in Oral Health Impact Profile-14 between patients with CLP and healthy controls (non-cleft group).
2. There are differences in Family Impact Scale between parents of children with CLP and parents of healthy children (non-cleft group).
3. There are differences in the perception of the quality of life between patients and their parents.
4. Age and sex influence the perception of the OHRQoL in both groups.

2. Materials and Methods

This cross-sectional study was approved by the Ethics Committee of Faculty of Medicine of University of Coimbra (Reference: CE-071/2020) (Appendix 5.1) in accordance with the Declaration of Helsinki. All participants, or parents when applicable, were instructed on the research and provided a written informed consent. This study was reported according to the STROBE (Strenthening the Reporting of Observational Studies in Epidemiology) guideline.

The sample size was based on previous studies that investigate the effects of orthodontic treatment on quality of life .[12] Prior data indicated an increase in OHRQoL score from 23.0 to 29.3 in patients undergoing orthodontic treatment. The initial calculated sample size was estimated of 48 individuals, with an 80% power in the detection of differences and 5% level of significance. Taking a 20% for loss to follow-up and drop outs into consideration, a final required sample size was 58 for each group. In the present work, the sample included a higher number of cleft and control individuals, allowing high confidence in the results.

During the data collection (July 2019 to February 2021), all patients in active orthodontic treatment for at least 6 months from the aforementioned Institution were invited to participate in this study. The sample was selected according to the following inclusion criteria:

- The Cleft group is composed by individuals from both sexes with cleft lip and palate undergoing orthodontic treatment in the Faculty of Medicine of University of Coimbra.
- The Control group is composed by individuals who attended the Faculty of Medicine of University of Coimbra for orthodontics care without cleft lip and palate condition.

The same number of patients was selected as control group, using randomized sampling. To ensure a reliable and unbiased comparison between groups, the control group was matched with the CLP group, regarding age and sex.

The exclusion criteria were patients with cognitive disorders, craniofacial syndromes, multiple dental loss, untreated dental caries, periodontal disease, severe facial trauma, chronic pain and patients who previously underwent orthodontic treatment.

Questionnaires

OHRQoL was assessed by 2 standardized instruments: Oral Health Impact Profile-14 (OHIP-14) (Appendix 5.2) and Family Impact Scale (FIS) (Appendix 5.3). Patients and parents were invited to complete the questionnaire after the routine orthodontic consultation in the waiting room and then return it in a sealed envelope to an individual not involved in the study.

The children's ORHQoL was assessed using the OHIP-14. OHIP-14 consists of 14 questions arranged over 7 domains: functional limitations, physical pain, psychosocial impact, physical limitation, psychological limitation, social limitation and disability. Participants were asked to rate the frequency of an event on a 5-point likert scale: never=0; once/twice=1; sometimes=2; often=3; every day/almost day=4. The sum of the responses allows the evaluation of total OHIP-14 scores and individual OHRQoL domain. A high OHIP-14 score corresponds a high negative impact on the OHRQoL. Participants were instructed to answer the questions without any support from their parents and if they have any doubt, clinical staff helps them. The age, sex and presence of cleft were also registered.

The FIS was used to evaluate the impact of a child's oral condition on family life. The FIS is composed of 14 questions into 4 domains: parental activities, parental emotions, family conflict and family finances. The questions have five Likert response options: never=0; once/twice=1; sometimes=2; often=3; every day/almost day=4. Summating all questions, ranging from 0 to 56, derives an overall FIS score. A higher FIS score indicates a greater impact of a child's oral condition on family.

Statistical analysis

Data were analysed using the Statistical Package for the Social Sciences, version 24.0 for Windows (SPSS Inc., Chicago, Illinois, USA). The significance level adopted for all analyses was 0.05. Descriptive statistics for the quantitative variables were obtained using mean, standard deviation values and minimum and maximum. Categorical variables were expressed as absolute and relative frequency.

The comparison between the groups was performed using the Mann-Whitney test for quantitative variables, when was verified a violation of the normality assumption through the Shapiro-Wilk test. The Fisher's exact test was used to verify statistical differences between groups for categorical variables.

The Spearman Rank Correlation Coefficient was used to correlate the results of the OHIP-14 and FIS questionnaires. Linear regression was performed to correlate sex and age with the OHIP-14 questionnaire.

3. Results

A total of 226 children or young adults (111 with cleft lip and palate and 115 healthy individuals) and their parents completed the OHIP-14 and FIS, respectively. Patients were in the age range of 8-27 years and homogenous individuals matched population was obtained regarding sex and age (Table 5.1). No statistically significant difference was found between both groups, sex and age variables (Table 5.2).

Table 5.1. Age and sex by each group.

	CLP Group (n=111)		Control Group (n=115)		
Year of Birth	**Female**	**Male**	**Female**	**Male**	**Total**
1994	1		1		2
1995	1	1	1	1	4
1996		1		1	2
1997	3	1	3	1	8
1998	1	3	1	3	8
1999	0	1	0	1	2
2000	3	2	3	2	10
2001	3	2	3	2	10
2002	4	4	4	4	16
2003	5	4	5	4	18
2004	5	8	5	6	24
2005	0	6	0	8	14
2006	5	8	5	7	25
2007	6	3	6	3	18
2008	1	3	1	3	8
2009	3	8	3	8	22
2010	1	7	1	12	21
2011	2	1	2	1	6
2012	1	0	1	0	2
2013	0	3	0	3	6
Total	**45**	**66**	**45**	**70**	**226**

Table 5.2. Demographic statistics for the sample studied

	Control Group (n=115)	CLP Group (n=111)	p
sex (M/F)	70/45 (60.9%/39.1%)	66/45 (59.5%/40.5%)	0.892§
age $\bar{x}$ ± sd (min/max) (min/max)	15.0±4.3 (7/36)	15.3±4.3 (7/26)	0.637£

§ Fisher's exact test; £Mann-Whitney; sd- standard deviation

Regarding the global questions, the mean score for quality of life according to the OHIP-14 was 9.4 ± 6.2 and 10.2 ± 7.2 for the control and CLP group, respectively. The parent-reported overall FIS score was 4.0 ± 5.4 for the control group and 6.7 ± 6.3 for the cleft group. No significant difference was found between groups in OHIP-14 score. Conversely, FIS score revealed a significant difference between the two groups evaluated (p<0.001) (Table 5.3). The distribution of values in OHIP-14 and FIS scores is shown in figure 5.1.

Table 5.3. Means scores of OHIP-14 and FIS

	Control Group (n=115)	CLP Group (n=111)	p
OHIP-14 $\bar{x}$ ± sd (min/max)	9.4±6.2 (0/29)	10.2±7.2 (0/40)	0.572£
FIS $\bar{x}$ ± sd (min/max)	4.0±5.4 (0/25)	6.7±6.3 (0/36)	<0.001£

£Mann-Whitney; sd- standard deviation

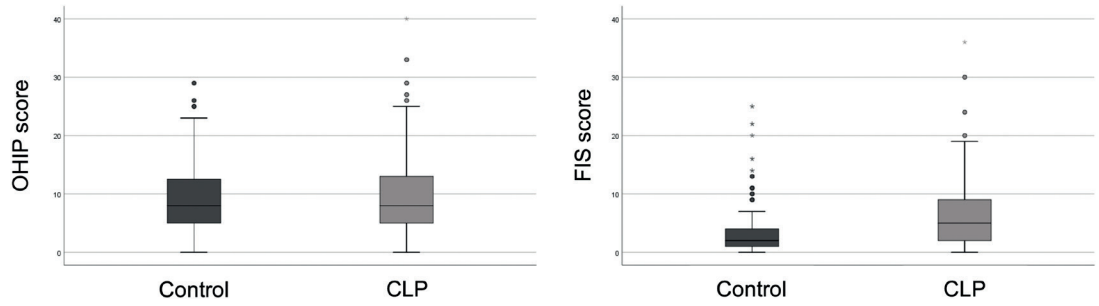

Figure 5.1. Distribution of values in OHIP-14 and FIS scores

There was a weak positive correlation between a patient's quality of life (OHIP-14) and a parent's quality of life (FIS) assessment. Additionally, it was also verified that control group ($r_{Spearmann}$=0.267, p=0.004) had a lower correlation than the CLP group ($r_{Spearmann}$=0.328, p<0.001). Figure 5.2 shows dispersion graphs of both groups.

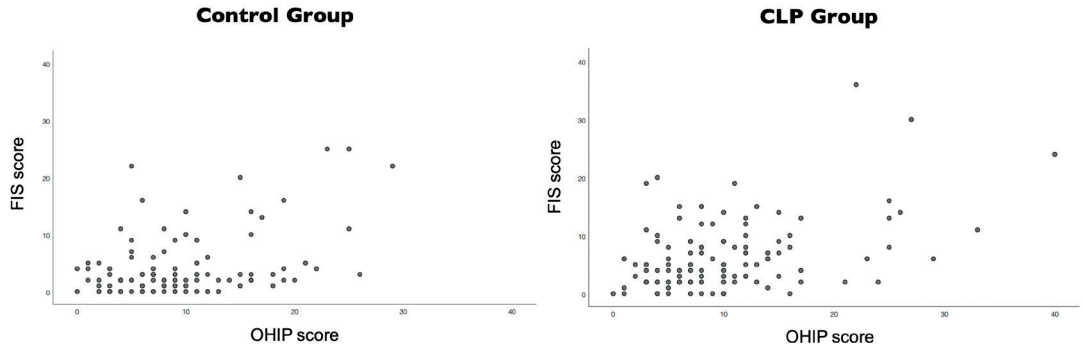

Figure 5.2. Correlation between patients' and parents' quality of life assessment

The analysis of the different OHIP-14 domains revealed that only the social limitation domain shows a significant difference between the two groups (p = 0.001), with CLP patients presenting the highest score. Regarding FIS score, the most affected dimensions were: family activities (p < 0.001), parental emotions (p = 0.001) and family conflict (p = 0.011). In all these three domains, parents with a cleft child had the highest scores than the control group. Table 5.4 shows the scores in the different OHIP-14 and FIS domains by the two groups.

Table 5.4. OHIP-14 and FIS answers distribution regarding the domains

		Control Group (n=115)	CLP Group (n=111)	p[£]
OHIP-14	Functional limitation	1.3 ± 1.0 (0/4)	1.6 ± 1.4 (0/6)	0.192
	Physical pain	3.2 ± 1.7 (0/8)	3.0 ± 1.6 (0/7)	0.507
	Psychosocial impact	1.3 ± 1.5 (0/6)	1.5 ± 1.9 (0/8)	0.904
	Physical limitation	1.5 ± 1.8 (0/8)	1.3 ± 1.6 (0/7)	0.253
	Psychological limitation	1.0 ± 1.4 (0/7)	1.1 ± 1.6 (0/7)	0.846
	Social limitation	0.8 ± 1.1 (0/4)	1.4 ± 1.5 (0/6)	0.001
	Disability	0.3 ± 0.7 (0/4)	0.3 ± 0.9 (0/4)	0.531
FIS	Family activities	2.0 ± 2.6 (0/12)	3.7 ± 3.2 (0/18)	<0.001
	Parental emotions	0.7 ± 1.6 (0/8)	1.3 ± 2.1 (0/12)	0.001
	Family conflict	0.7 ± 1.6 (0/7)	1.2 ± 2.0 (0/9)	0.011
	Family finances	0.6 ± 0.8 (0/4)	0.4 ± 0.9 (0/3)	0.051

[£]Mann-Whitney

The analysis of the correlation between age or sex on quality of life impacts showed that both variables do not affect the perception of OHIP-14 score (sex: p = 0.170; age: p = 0.406).

4. Discussion

This study evaluated the quality of life in 226 children with and without CLP and their parents with two validated indexes (OHIP-14 and FIS) during orthodontic treatment. It was found that children had a similar OHRQoL but the family impact was highest in parents of cleft children.

Malocclusion has been reported to play a negative role on quality of life.[8] Since CLP patients have usually malocclusion due to dental anomalies and abnormal craniofacial development, it would be expected that CLP patients presented worse scores of OHRQoL.[2] Despite that, this study verified no significant difference in OHIP-14 score, which is in line with previous studies.[22-23] Aravena et al. reported similar OHRQoL on CLP and control children despite the cleft group had a lower quality of life score concerning speech items.[23] Additionally, the current study included cleft patients undergoing orthodontic treatment, demonstrating that OHRQoL score remains similar between groups. In the literature, a positive effect after orthodontic treatment in healthy and CLP patients was reported.[21,24] Chen et al. showed that completed orthodontic treatment had a positive effect on the quality of life, especially when orthodontic surgical treatment was performed.[24]

The social limitation was the only OHIP-14 domain with a significant difference between the two groups ($p=0.001$), with CLP patients presenting the highest score. This result is in accordance with the study of Sundell et al. which verified that the 10-year-old children with CLP perceived lower OHRQoL than the non-cleft controls.[25] Khoun et al. found that the cleft group had a greater impact on emotional and social well-being compared to the others study groups (severe caries; malocclusion; and, dentine caries).[26] Several explanations for this finding were given namely, CLP children feel less acceptance by peers due to the facial appearance and hypernasal speech; CLP children have less ability to maintain relationships; and, CLP children may have fewer experiences of positive group feeling.[25]

The family's perspective should also be assessed because chronic illness, like cleft lip and palate, inevitably impacts the day-to-day functioning since health care interventions will lead to family needs and concerns.[27] Family activities, parental emotions and family conflict were identified as significant factors negatively affecting parents OHRQoL in this total sample. Previous studies pointed out some factors that may explain the impact on quality of life of caregivers namely, repeated consultations, financial implications, parental time off work, less time for other family members, stigmatization and

peer victimization of their child, and the impact of orthodontic appliances in speech, mastication and social interaction of the child.[27-28] On the other hand, the family finances domain did not show a significant difference between the two groups (p = 0.051). Cuyper et al. found that parents of non-syndromic children had more family conflicts, suggesting that having a cleft child entails financial implications and decreased participation in social activities.[28] These distinct findings may be explained by this cross-sectional study having a sample recruited from a university hospital environment where the treatments provided are supported by the National Health Service. Therefore, this sample may be limited in representing the population in this domain, as the recruitment to the university hospital depends on the eligibility for funding.

Concerning a patient's quality of life (OHIP-14) and a parent's quality of life (FIS) assessment, it has been verified a weak positive correlation. Parents of cleft children had a poorer OHRQoL compared to what was perceived by their children. Several reasons for this finding have been suggested, namely the overestimation of the cleft implications in children social integration at early ages, guilty feelings, and better understanding of the impact of cleft on children´s development, which can lead to a higher burden of concerns.[22,29] Additionally, Imani et al. suggested that parents of CLP children under orthodontic treatment are more vulnerable due to their previous adverse experiences throughout the treatment of their children.[30]

Finally, no significant difference was found regarding the correlation between age (p = 0.406) or sex (p = 0.170) on quality of life impacts. This not corroborated earlier studies that showed that the higher the age the higher the impact on quality of life and that females generally have a greater impact on OHRQoL.[16] The results obtained in this study can be explained by the homogenous age and sex group distribution of the sample. Recently, Agnew et al. demonstrated no difference in FIS score by age, sex or whether a child had started orthodontic treatment.[27]

This study presents some limitations that need to be discussed. Firstly, the scores used were not developed to evaluate craniofacial deformities. CLP patients have several problems that are not directly assessed by the scores used, such as facial and speech features.[9] Second, the answers to the questionnaire can be influenced by others aspects of life that are not necessarily related to craniofacial deformity. However, the scores (OHIP-14 and FIS) used in this study are widely used in dentistry to measure the quality of life, permitting to compare the findings of this study with other studies. Moreover, these scores had validation in the native language of this sample and it has previously been shown that OHIP-14 is a good method to measure OHRQoL due to its simplicity and good discriminative properties in patients with simple and complex treatment (eg dentofacial deformities).[14] Third, no distinction between patients regarding the severity of the cleft or malocclusion was performed, which could potentially introduce some bias. Nevertheless, Sundell et al. reported no differences between cleft type and overall mean score.[25]

In spite of these limitations, this study presents some strengths such as adequate sample power with a homogenous distribution, allowing to reduce the bias of the analyzed data. This study presents novel insights into the impact of orthodontic treatment in quality of life of patients and their families, which may contribute to the communication among physicians, patients and parents/caregivers. Further studies should establish greater insight into specific factors influencing quality of life such as the severity of cleft or malocclusion. Moreover, the effects of various phases of orthodontic treatment should be studied.

5. Conclusion

Undergoing orthodontic treatment had a similar impact on the overall quality of life in CLP patients and non-cleft patients. Parents of cleft children had a poorer OHRQoL compared to what was perceived by their children and parents of non-cleft children. No significant difference was found regarding the age and sex of the child.

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VI

Chapter VI

Regenerative strategies in cleft palate: an umbrella review

1. Introduction

Cleft lip and palate (CLP) is a craniofacial malformation with a prevalence in newborns of 14 per 10 000 live births worldwide.[1] Although not yet fully understood, several genetic and environmental risk factors are associated with CLP.[2] Smoking, gestational diabetes, and genetic association with the IRF6, VAX1, and PAX7 genes are consistently reported.[3-4] Most CLP patients have several hearing, feeding, speaking, and dentofacial development complications, leading to a long-term impact on the patient's facial anatomy and self-esteem.[5-6] Additionally, new evidence suggests a common basis for orofacial cleft and cortical interneuronopathy, supported by cellular and molecular Central Nervous System (CNS) alterations in these patients.[7]

The CLP patients treatment requires a coordinated interdisciplinary team that considers the patient's and family's needs.[8-9] One of the endpoints in CLP care is a secondary bone graft, which presents several benefits such as support of unerupted teeth that will erupt into the bone graft, support of alar bases (promoting nasal and lip symmetry), closure of oronasal fistulas and cleft maxillary segments stabilization.[10-12] The bone graft is usually performed when the canine has one-half to two-thirds root formed. However, a recent systematic review (SR) that compares bone graft performed early at approximately 5-6 years and at the conventional time at 9-11 years did not found significant differences between the two protocols.[13] Since its introduction by Boyne and Sands in 1972, autologous bone graft using cancellous bone remains the gold-standard until today.[14] Cancellous bone grafts have abundant osteogenic surface cells, which allow the new bone formation.[15] The origin of autogenous bone graft depends on numerous factors, namely, the surgeon's experience, the volume of alveolar defect, and the morbidity of the harvest area.[16] Typically, an autologous bone graft from the iliac crest (ICBG) is the conventional donor site.[17] Still, others are suggested with less morbidity and lower bone resorption rate, such as cranial bone, mandibular symphysis and tibia.[18-20] Nevertheless, autologous bone graft has several limitations, such as limited donor supply and/or self-renewal capacity, operative time, costs and donor site morbidity.[21] Furthermore, it is also reported that bone resorption can be approximately 40% after one year of bone graft, which may increase the need for reintervention.[18]

Therefore, tissue regeneration presents as a new emerging and alternative approach to conventional bone grafts in cleft patients. Besides the bone regeneration capacity, these strategies can modulate inflammation and enhance the healing process.[22] Many substitute materials or agents alone or combined with autogenous bone have been suggested to regenerate bone, such as growth factors like bone morphogenic protein 2 (BMP-2), platelet-rich fibrin, bone scaffolds with or without cell treatment (eg mesenchymal stem cells (MSCs) or osteoblast), biocomposites (eg calcium phosphate and hydroxyapatite) and haemostatic agents (eg fibrin glue).[16,23-29] Stem cell-based therapies have been explored based on several stem cell types: bone-marrow stem cells, adipose-derived stem cells, umbilical cord mesenchymal stem cells, and others.[30-31] Besides the direct effect on bone regeneration, the activation of resident stem cells can present a broader impact, for instance, on the CNS alterations previously described.[7] For this specific purpose, adult neural stem cells (NSCs) are the main cell population involved. These cells are maintained through lifetime on the lateral ventricles and the hippocampus, have a multipotent capacity, long-term stemness and can produce neurons and microglia cells.[32] In adults, they are mainly in a quiescent state, characterized by an increased expression of Sox9, Id2, Id3, Id4, Vcam, Cdh2, Klf9 and Lrig1.[33] When stimulated, NSCs can become active and contribute to tissue regeneration.[34] It must be pointed out that evidence regarding some of these therapeutic methods is often weak due to the lack of standardization across studies.

Despite a recently renewed focus on bone graft materials, an overall synthesis and appraisal of these reviews is still lacking.[35] To clarify it, we propose to conduct an umbrella review to assess the clinical effectiveness of regenerative strategies on the treatment of secondary bone graft in cleft patients, which would be advantageous for readership since it synthesizes what we know in a single paper. Therefore, this umbrella review aims to answer the following focused question: "Is the regenerative capacity of new bone graft strategies more effective than the gold-standard?"

2. Materials and Methods

Protocol registration

This review was registered in the International prospective register of systematic reviews (PROSPERO) with provisional number 240534. This study was conducted according to the Cochrane and PRISMA guidelines for systematic reviews.[36-38]

Review question

The purpose of this umbrella review was to assess the clinical effectiveness of regenerative strategies on cleft patients treatment, with the following PICO question:

Population—cleft palate patients (non-syndromic cleft lip and/or palate patients (unilateral and bilateral) of all ages that underwent regenerative strategies as part of their treatment);

Intervention—undergoing treatments approaches with regenerative strategies (all available treatment approaches for cleft palate closure: conventional autologous graft from different origins, alloplastic material, platelet-rich fibrin, platelet-rich plasma, resorbable collagen sponge, bovine-derived hydroxyapatite, allogeneic bone material, demineralized bone matrix, acellular dermal matrix and human bone morphogenetic protein 2);

Comparison—different available regenerative strategies;

Outcome—bone regeneration.

Eligibility criteria

The included studies were all SRs of randomized trials, non-randomized controlled trials and case control studies. Studies that included regenerative strategies but evaluated other outcomes, such as quality of life, feeding problems, phonetics problems, assessment method, velopharyngeal function, were excluded. Case reports, case series and literature reviews were also excluded.

Search strategy

A standardized literature search was performed in electronic bibliographic databases (MEDLINE via PubMed, Web of Science databases, Cochrane Library, Scopus and EMBASE), up to 27 February 2021. The search strategy can be seen in table 6.1.

The search for unpublished articles in the grey literature was carried out through the websites Proquest (https://www.proquest.com) and OpenGrey Europe (https://opengrey.eu).

Table 6.1. Search keys in various databases.

Databases	Search keys
PubMed	"Cleft palate"[Mesh] OR "cleft palate" OR "oral cleft*" OR "orofacial cleft*". Filters: systematic reviews
Web of Science (all databases)	TS=("cleft palate" OR "oral cleft*" OR "orofacial cleft*") AND TS=("systematic review")
Cochrane Library	#1 MeSH descriptor: [Cleft palate] explode all trees #2 "oral cleft*" #3 "orofacial cleft*"
Scopus	"cleft palate" OR "oral cleft*" OR "orofacial cleft*" AND "systematic review"
EMBASE	('cleft palate'/exp OR 'cleft palate' OR 'oral cleft*' OR 'orofacial cleft*') AND 'systematic review'

The reference lists of the relevant articles were manually searched to explore additional studies.

Studies selection and data collection

The search and study selection were carried out by two reviewers (IF and ABP). If the two reviewers could not agree on a certain study's eligibility, another reviewer resolved disagreements (CMM). The articles were screened based on the titles and abstracts according to the eligibility criteria by the two independent reviewers mentioned above, in duplicate. Subsequently, full texts were screened for potential inclusion and disagreements were resolved through mediation with the third reviewer.

From each included study, the authors extracted the following information: authors and publication data, design of studies included and their number, sample characteristics (age of participants, intervention and comparative unit), primary outcome, and evaluated parameters in the study. If present, the meta-analysis model used, effect size with a 95% confidence interval, I^2 statistic, heterogeneity, and GRADE evidence.

A descriptive summary of each study main findings was performed.

Quality assessment

The selected studies qualitative assessment was performed using the Assessment of Multiple Systematic Reviews (AMSTAR 2) (https://amstar.ca/mascripts/Calc_Checklist.php) checklists. AMSTAR2 checklists contain several questions directed only to SRs under evaluation (Appendix 6.1).[39,40] Two

reviewers (IF and ABP) performed the quality assessment of the studies in duplicate and independently, categorizing them as: high quality if none or only one of the parameters is weak; moderate quality if more than one parameter is weak; and poor quality if there are several weak parameters or a major failure. Three other reviewers (CMM, EC and FV) also independently assessed the studies' quality, and, if in disagreement with the initial evaluation, this point was discussed together.

Statistical analysis

A random effects meta-analysis over the standardized mean difference between ICBG and BMP-2 was conducted whenever at least three SRs were reporting the same synthesis measure. A synthetic standardized effect size was computed using the total number of subjects included in the previous meta-analysis studies, and its correspondent 95% confidence intervals and p-value for the comparison between ICBG and BMP-2 along with forests plots and funnel plots. The Egger regression and Begg-Mazumdar test were applied to assess publication bias regarding the dependency of outcome measures from previous meta-analysis variability. Publication bias was also evaluated for the three meta-analyses performed.

The analysis was performed through the 'metafor' package of R, version 4.0.3 implemented in R Studio, version 1.3.1093 and was analyzed at a 5% significance level.

3. Results

Studies selection

The flow chart for this umbrella review can be seen in figure 6.1. The search in the different databases resulted in 1317 articles, with no paper retrieved from grey literature or from the manual search. After the articles were screened by title and abstract, 20 full-text articles were assessed for eligibility. Full-text screening led to the exclusion of 11 articles due to several reasons: 1 case-report, 2 literature reviews, 4 with different interventions or outcomes, 4 with other measures of bone regeneration and 1 poster abstract. Nine SRs were included in the qualitative analysis and five in the quantitative analysis (meta-analysis).

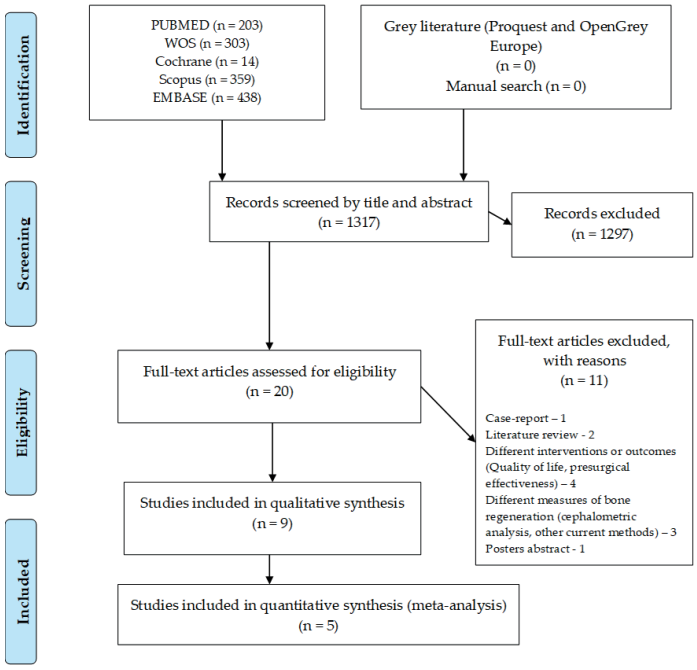

Figure 6.1. Flow chart for included systematic reviews.

Characteristics of the included reviews

The characteristics and results of the included reviews are presented in table 6.2 and 6.3.

This umbrella review includes 9 systematic reviews comprising 56 RCT, 61 Non-RCT and 9 case-control cases. Five SR were registered in PROSPERO and four do not report any registration. Most SR used the Cochrane (n=4) ROB tool, the others used MOOSE / Strobe (n=1), Oxford Centre for Evidence-Based Medicine (n=1) or Newcastle-Ottawa (n=1), and two studies do not report any assessment. The evidence quality was considered high in 15, moderate in 11 and low in 39 of the studies included in the SRs. A SR that used the Newcastle-Ottawa tool was classified as level 6 and 7.

The included patients age ranges from over five years old to adolescence (16 years old). However, in 4 reviews the age parameter is not reported.

In all reviews, the comparison or control technique used was the ICBG, which is considered the gold-standard. The interventions or new bone regeneration strategies were diverse, although BMP-2 was used in most studies. The other strategies were: bone substitutes (Bioglass, β-tricalcium phosphate, Hidroxyapatite); cells (osteoblasts); other biomaterials (Plaquet rich plasma - PRP, demineralized dentinal matrix - DDM, demineralized bone matrix - DBM, acellular dermal matrix - ADM, Bio-guide Membrane, Periosteum, deproteinized bovine bone - DBB) with ICBG; or autogenous bone grafts from other sources (cranium, rib, tibia, mandible, symphysis and calvarium grafts).

The primary outcome evaluated was the volume, filling, heigh, rate and density of bone regeneration. The secondary outcomes evaluated were the failure rate, the postoperative infection, the persistence rate of the fistula and the length of hospital stay.

Quality of the included reviews

The quality of the included reviews evaluated by the AMSTAR2 tool can be seen in table 6.4. All reviews presented the PICO question except one.[41] Successful registration was always carried out except in one review[41] and in another in a partial form[16]. The inclusion criteria were omitted in some reviews since the PICO issue was already explained.[16,42-44] The search was only fully explained in two reviews.[24,43] However, data selection and extraction were carried out in duplicate, except in one review.[16] The list of excluded studies is not presented in a single review. The description of the included studies was not done in one study.[41] In a review, the risk of bias assessing was not performed.[45] The funding of included studies was not reported in most of the reviews.[16,24,42,45-47] Three reviews did not presented meta-analysis and consequently do not have combined statistical results[16,41,44], risk of bias effects on the statistical combination[16,41,44-45] and risk of bias in the discussion[16,41,44-45,47]. Heterogeneity is discussed in some studies[24,41-44,47]. Publication bias was presented in only one review.[43] The study funding and the conflict of interests report were not referred to in one review.[16] Thus, with the application of the AMSTAR 2 tool criteria, 4 reviews were considered to be of low quality[16,41,44-45], 4 were considered to be of moderate quality[24,42,46-47] and 1 was considered to be of high quality[43].

Table 6.2. The characteristics of the included systematic reviews.

Author year	Design	Registration	No. of trials and design	ROB tool	Quality of evidence	Age of participants	Intervention	Comparison unit
Da Rosa et al.[46] 2019	SR/MA	Pros	RCT (4) RS (5) PS (1)	Cochrane guidelines	LROB (10)	7-16.4 years	rhBMP-2	ICBG
Uribe et al.[47] 2019	SR/MA	Pros	RCT (4) N-RCT (1)	Cochrane RBAT	Low-quality evidence (GRADE): HROB (5)	9.5 – 16.15 years	rhBMP-2 scaffold	ICBG
Wu et al.[45] 2017	SR/MA	Pros	RCT (14) N-RCT (26)	Oxford Centre for Evidence-Based Medicine 2011 Levels of Evidence	Best Evidence Synthesis Moderate Methodological Quality (7) Low Methodological Quality (18)	NR	1.Bone substitute materials (Bioglass, β-TCP, HA, Osteoblasts, BMP-2) 2.Supplementary materials (PRP, DDM, DBM, ADM, Bio-guide, Membrane, Periosteum, DBB) + ICBG 3.Autogenous bone grafts (cranium, rib, tibia and mandible grafts)	Iliac crest bone graft
Khojasteh et al.[16] 2015	SR	NR	CS (1) CR (3) CT (14)	Quality assessment (NR Tool)	Quality of Evidence - NR	NR	Cell group Osteoblasts (maxilla) MSC (Bone narrow) Growth factor rhBMP-2 Plaquet rich plasma Plaquet rich fibrin	ICBG Autogenous bone graft
van Hout et al.[41] 2011	SR	NR	RCT (2) CRR (1)	-	-	Children and adolescents	BMP-2	Autologous bone graft
Xiao et al.[42] 2020	SR/MA	NR	Case-control(9)	Newcastle-Ottawa scale	NOS 7 (5) NOS 6 (4)	NR	BMP-2	ICBG
Scalzone et al.[43] 2019	SR	Pros	RCT (4)	Cochrane	GRADE- low High risk (2) Unclear risk (2)	>5 years old	rhBMP-2	ICBG
Guo et al.[44] 2011	SR	NR	RCT (2)	Cochrane	High risk (2)	Children and adolescents	rhBMP-2 Iliac bone grafting + fibrin glue applied to the bone graft	ICBG
Kamal et al. [24] 2018	SR/MA	Pros	RCT (12) PS (10) RS(13)	MOOSE STROBE	Low risk (6) Moderate risk (9) High risk (11)	NR	Autogenous bone graft (iliac crest, tibia, mandibular symphysis, calvarium)	Growth factors Improved scaffolds and cell treatment Biocomposites and haemostatic agents

ADM, acellular dermal matrix; BMP-2- bone morphogenetic protein–2; CR- Case report; CS- Case series; CT- Clinical trial; DBB, deproteinized bovine bone; DBM, demineralized bone matrix; DDM, demineralized dentinal matrix; HA- hydroxyapatite; HROB- high risk of bias; ICBG- Iliac cancellous bone graft; LROB – Low risk of bias; MSC- Mesenchymal stem cell; NOS- Newcastle-Ottawa scale; N-RCT- non- randomized controlled trial; NR- not registered; PRF- Plaquet rich fibrin; Pros- Prospero; PRP- Platelet-rich plasma; PS- prospective study; RCR- Retrospective controlled review; RCT- randomised controlled trial; rhBMP-2- Recombinant human bone morphogenetic protein–2; RS- retrospective study; SR- systematic review; SR/MA, systematic review and meta-analysis; β-TCP- β-tricalcium phosphate.

Table 6.3. The main results of the included systematic reviews

Author year	Results
Da Rosa et al.[46] 2019	(5 RCT) Total(95%CI): BMP (54); ICBG (54); Weight 100% Std.Mean Difference 0.07 [-0.41, 0.56]; Heterogeneity: Tau^2=0.14; Chi^2 = 10.81, df = 8 (P = 0.21); I^2 = 26%; Test overall effect: Z= 0.30 (P=0.77). (5 RCT) Total(95%CI): BMP (95); ICBG (80); Weight 100% Std.Mean Difference 0.24 [-0.32, 0.80]; Heterogeneity: Tau^2=0.38; Chi^2 = 18.86, df = 7 (P = 0.009); I^2 = 63%; Test overall effect: Z= 0.83 (P=0.41).
Uribe et al.[47] 2019	(3 RCT) Total(95%CI): BMP (16); Iliac crest (17) Weight 100% Std.Mean Difference -208.76 [-253.59, -163.93]; Heterogeneity: Chi^2 = 0.38, df = 2 (P = 0.83); I^2 = 0%; Test overall effect: Z= 9.13 (P<0.00001).
Wu et al.[45] 2017	BMP-2 (4RCT) Total(95%CI): BMP (54); ICBG (46); Weight 100% Std.Mean Difference -2.16 [-10.10, 5.78]; Heterogeneity: Tau^2= 34.44; Chi^2 = 7.84, df = 3 (P = 0.05); I^2 = 62%; Test overall effect: Z= 0.53 (P=0.59). ADM+ICB (4RCT) Total(95%CI): ADM+ICB (121); ICBG (171); Weight 100% Std.Mean Difference 1.34 [1.15,1.55] Total events: ADM+ICB (98); ICBG (104); Heterogeneity: Chi^2 = 4.46, df = 3 (P = 0.22); I^2 = 33%; Test overall effect: Z= 3.80 (P=0.0001). Cranium Grafts Total(95%CI): Cranium grafts (117); ICBG (193); Weight 100% Std.Mean Difference 0.86 [0.76,0.97] Total events: Cranium grafts (92); ICBG (173); Heterogeneity: Chi^2 = 4.18, df = 3 (P = 0.24); I^2 = 28%; Test overall effect: Z= 2.53 (P=0.01). Rib Grafts Total(95%CI): Rib grafts (38); ICBG (135); Weight 100% Std.Mean Difference 0.55 [0.37,0.83] Total events: Rib grafts (15); ICBG (97); Heterogeneity: Chi^2 = 3.52, df = 1 (P = 0.06); I^2 = 72%; Test overall effect: Z= 2.87 (P=0.004).
Khojasteh et al.[16] 2015	Descriptive evaluation Cell group Application of stem cells in alveolar cleft patients resulted in less than 50% of new bone formation, except in one case report, which was remarkable for 79.1% bone formation. MSCs seeded on DBM with calcium sulfate achieved 34.5% of new bone filling. Growth factor Application of BMP-2 with collagen led to 71.7% new bone filling. In one study, 90.9% of fistula closure was reported in cleft patients after the use of PRGF. PRP showed 71.27% bone filling compared to 47.47% in the control group, with 26.5% of secondary bone loss compared to 35.5% in the control group.
van Hout et al.[41] 2011	Descriptive evaluation Dickinson et al. reported 95% of bone formation in the rhBMP-2 group compared to the 63% control group. Alonso et al. and Herford et al. reported less bone formation in the rhBMP-2 group compared to the control group (5.8% and 7%).

Author year	Results
Xiao et al.[42] 2020	Bone graft filling rate Total(95%CI): BMP-2 (54); ICBG (46); Weight 100% Std.Mean Difference -0.05 [-0.79,0.69]; Heterogeneity: Tau^2=0.30; Chi^2 = 6,79; df = 3 (P = 0.08); I^2 = 56%; Test overall effect: Z= 0.13(P=0.90). Bone graft volume Total(95%CI): BMP-2 (24); ICBG (16); Weight 100% Std.Mean Difference -0.42 [-1.44,0.60]; Heterogeneity: Tau^2=0.41; Chi^2 = 4.02; df = 2 (P = 0.13); I^2 = 50%; Test overall effect: Z= 0.8(P=0.42). Bone graft height Total(95%CI): BMP-2 (14); ICBG (14); Weight 100% Std.Mean Difference -21.38 [-23.00,-19.76]; Heterogeneity: Chi^2 = 4.88; , df = 1 (P = 0.03); I^2 = 80%; Test overall effect: Z= 25.81(P<0.00001). Bone graft density Total(95%CI): BMP-2 (37); ICBG (38); Weight 100% Std.Mean Difference -0.43 [-0.79,1.64]; Heterogeneity: Tau^2= 0.56; Chi^2 = 3.31; df = 1 (P = 0.07); I^2 = 70%; Test overall effect: Z= 0.69(P=0.49). Failure Rate Total(95%CI): BMP-2 (316); ICBG (320); Weight 100% Std.Mean Difference 0.02 [-0.03,0.06]; Total events: BMP-2 (316); ICBG (320); Heterogeneity: Tau^2= 0.00; Chi^2 = 1.91; df = 4 (P = 0.75); I^2 = 0%; Test overall effect: Z= 0.67(P=0.50). Infection after bone graft Total(95%CI): BMP-2 (294); ICBG (262); Weight 100% Std.Mean Difference 0.20 [0.05,0.73]; Total events: BMP-2 (3); ICBG (11); Heterogeneity: Chi^2 = 0.41; df = 1 (P = 0.52); I^2 = 0%; Test overall effect: Z= 2.44(P=0.01). Rate of oronasal fistula Total(95%CI): BMP-2 (45); ICBG (31); Weight 100% Std.Mean Difference 0.41 [0.06,2.63]; Total events: BMP-2 (1); ICBG (3); Heterogeneity: Chi^2 = 1.15; df = 1 (P = 0.28); I^2 = 13%; Test overall effect: Z= 0.94(P=0.35). Operative time Total(95%CI): BMP-2 (48); ICBG (34); Weight 100% Std.Mean Difference -3.64 [-7.35,0.06]; Heterogeneity: Tau^2= 6.68; Chi^2 = 15.06; df = 1 (P = 0.0001); I^2 = 93%; Test overall effect: Z= 1.93(P=0.05) length of hospital stay. Total(95%CI): BMP-2 (21); ICBG (27); Weight 100% Std.Mean Difference -1.97 [-2.41,-1.53]; Heterogeneity: Chi^2 = 45.18; , df = 1 (P < 0.00001); I^2 = 98%; Test overall effect: Z= 8.74(P < 0.00001).
Scalzone et al.[43] 2019	Bone graft Volume after 6 months Difference means -14.410; Standard error 4.072; Variance 16.585; Lower limit -22.392; Upper limit -6.428; Z-value -3.538; p-value 0.000; (MD − 14.410; 95% CI − 22.392 to − 6.428; p = 0.000). Bone graft Volume after 1 year Difference means 6.227; Standard error 11.324; Variance 128.234; Lower limit -15.967; Upper limit -28.422; Z-value -0.550; p-value 0.582; (MD 6.227; 95% CI − 15.967 to 28.422; p = 0.582). Bone graft Volume after 1 year considering patient's age Standard Difference in means -0.493; Standard error 0.386; Variance 0.149; Lower limit -1.249; Upper limit -0.263; Z-value -1.278; p-value 0.201; (MD 30.000; 95% CI 11.593 to 48.407; p = 0.001); Dickinson's data (MD − 0.493; 95% CI − 1.249 to 0.263; p = 0.201). Bone graft height 6 months Difference means -18.737; Standard error 12.665; Variance 160.413; Lower limit -43.560; Upper limit 6.087; Z-value -1.479; p-value 0.139; (MD − 18.737; 95% CI − 43.560 to 6.087; p = 0.139). Bone graft height 1 year Difference means -4.401; Standard error 13.386; Variance 179.172; Lower limit -30.636; Upper limit 21.834; Z-value -0.329; p-value 0.742; (MD − 4.401; 95% CI − 30.636 to 21.834; p = 0.742). Bone graft height after 1 year considering patient's age Standard Difference in means -6.523; Standard error 6.209; Variance 38.557; Lower limit -18.694; Upper limit 6.647; Z-value -1.051; p-value 0.293; (MD − 6.523; 95% CI − 18.694 to 5.647; p = 0.293). Length of hospital stay Standard Difference in means -1.146; Standard error 0.511; Variance 0.261; Lower limit -2.147; Upper limit -0.145; Z-value -2.244; p-value 0.025; (MD − 1.146; 95% CI − 2.147 to − 0.145; p = 0.025).

Author year	Results
Guo et al.[44] 2011	Descriptive analyses Traditional iliac bone graft versus artificial bone graft materials (+rhBMP-2). BMP-2 group (n = 9) had a score 0.9 point higher when compared to the iliac grafting group (n = 12) (mean difference (MD) -0.90; 95% CI -1.16 to -0.64). After follow-up, the mean value of nasal alar base augmentation was 2.2 in the BMP-2 group (n = 9) compared with 2.0 in the iliac grafting group (n = 12), with no significance between the two groups (MD -0.20; 95% CI -0.41 to 0.01). Traditional iliac bone graft versus traditional iliac bone graft plus fibrin glue The average amount of graft resorption varied from 62.25%in the control group to 29.72% in the intervention group. The mean coronal bone volume was reported as 42.62 cm^3 greater in the intervention group (64.32 cm^3) when compared with the control group (21.70 cm^3) (MD -42.62; 95% CI -64.25 to - 20.99), and mean coronal bone density was 150.89 HU less in the control group (245.68 HU) than intervention group (396.57 HU) (MD -150.89; 95% CI -298.33 to -3.45). Regarding complications, dehiscence in the intervention group (infection in wound (RR 0.31; 95% CI 0.01 to 7.02); dehiscence (RR 2.79; 95% CI 0.33 to 23.52)).
Kamal et al. [24] 2018	Reduction in postoperative volume using autogenous bone graft Overall (Random effects): hedge'g SMD -1.91; Lower -2.25; Upper -1.57; p-value 0.000.; Heterogeneity: q-value =105.7; df=24, p-value<0.001; I^2 = 77,3% (overall SMD =-1.91, 95% CI: -2.25 to –1.57, p<0.001, I^2=77,3%). Reduction in postoperative volume using tissue-engineered bone substitutes Overall (Random effects): hedge'g SMD -1.95; Lower -2.64; Upper -1.27; p-value 0.000.; Heterogeneity: q-value =28.8; df=9, p-value 0.001; I^2 = 68.7% (overall SMD =-1.95, 95% CI: -2.64 to – 1.27, p<0.001, I^2=68,7%). Subgroups analysis of studies using autogenous bone graft Iliac crest- nr studies: 22; nr subjects: 371; hedges'g SMD (95% CI): -1.78(-2.11 to –1.45); SE 0.169; Within group p value <0.001. Mandibular symphysial- nr studies: 1; nr subjects: 32; hedges'g SMD (95% CI): -3.12(-3.95 to –2.28); SE 0.426; Within group p value <0.001. Cranial- nr studies: 1; nr subjects: 10; hedges'g SMD (95% CI): -3.22(-4.75 to –1.7); SE 0.777; Within group p value <0.001. Tibial - nr studies: 1; nr subjects: 9; hedges'g SMD (95% CI): -2.46(-3.74 to –1.18); SE 0.654; Within group p value <0.001. p value between groups- 0.009. Subgroups analysis of studies using tissue-engineered bone substitutes Growth factors- nr studies: 6; nr subjects: 49; hedges'g SMD (95% CI): -2.34(-3.39 to –1.28); SE 0.540; Within group p value <0.001. Improved scaffolds and cell treatment- nr studies: 2; nr subjects: 11; hedges'g SMD (95% CI): -1.82(-3.27 to –0.37); SE 0.742; Within group p value 0.014. Biocomposites and haemostatic agentes- nr studies: 2; nr subjects: 11; hedges'g SMD (95% CI): -1.20(-2.35 to –0.05); SE 0.587; Within group p value 0.041; p value between groups 0.362.

ADM, acellular dermal matrix; df – degrees of freedom; BMP-2- bone morphogenetic protein–2; CI – confidence interval; DBM, demineralized bone matrix; ICBG- Iliac cancellous bone graft; MD – mean difference; MSC- mesenchymal stem cell; nr- number; PRF- plaquet rich fibrin; Pros- prospero; PRP- platelet-rich plasma; PS- prospective study; p – p-value; RCT- randomised controlled trial; rhBMP-2- recombinant human bone morphogenetic protein–2; SMD – standardized mean difference; SE - standard error.

Table 6.4. Quality assessment of the included reviews, using the AMSTAR2 tool.

Author/year	PICO	Protocol	Inclusion criteria	Comprehensive search	Duplicate in selection	Duplicate in data extraction	List of excluded studies	Description of included studies	Assessing risk of bias	Funding of included studies	Results of statistical combination	ROB effect on the statistical combination	ROB in the discussion	Discussion for the heterogeneity	Publication bias	Author's funding and COF reporting	Overall quality
Da Rosa et al. 2019 [46]	Yes	Yes	Yes	Partial Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	No	Yes	No	No	Yes	Moderate
Uribe et al. 2019 [47]	Yes	yes	Yes	Partial Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	No	Yes	No	Yes	Moderate
Wu et al. 2017 [45]	Yes	yes	Yes	Partial Yes	Yes	Yes	Yes	Yes	No	No	Yes	No	No	No	No	Yes	Low
Khojasteh et al. 2015 [16]	Yes	Partial Yes	No	No	No	No	No	Partial Yes	Partial Yes	No	No meta-analysis	No meta-analysis	No	No	No meta-analysis	No	Low
van Hout et al. 2011 [41]	No	No	Yes	Partial Yes	Yes	Yes	Partial Yes	No	Yes	Yes	No meta-analysis	No meta-analysis	No	Yes	No meta-analysis	Yes	Low
Xiao et al. 2020 [42]	Yes	Yes	No	Partial Yes	Yes	Yes	Partial Yes	Partial Yes	Yes	No	Yes	Yes	Yes	Yes	No	Yes	Moderate
Scalzone et al. 2019 [43]	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	High
Guo et al. 2011 [44]	Yes	Yes	No	Partial Yes	Yes	Yes	Yes	Yes	Partial Yes	Yes	No meta-analysis	No meta-analysis	Yes	Yes	No meta-analysis	Yes	Low
Kamal et al. 2018 [24]	Yes	Yes	Yes	Yes	Yes	Yes	Partial Yes	Partial Yes	Yes	No	Yes	No	Yes	No	No	Yes	Moderate

PICO – population, intervention, comparison, and outcome; ROB – risk of bias; COF – conflict of interests.

Synthesis of the best evidence

Bone formation volume analysis

The quantitative analysis of previous meta-analysis synthetic measures was possible using three systematic reviews (Rosa et al. 2019, Uribe et al. 2019 and Xiao et al. 2020)[42,46-47] and has presented null heterogeneity between previous synthetic measures (I^2 = 0.00%). Besides this, the random effects model was still applied due to the number of studies in analysis, and we found that the global standardized mean difference between ICBG and BMP-2 was not statistically significant (p = 0.704) and was estimated to be 0.08mm^3 + 0.22mm^3 (95%CI: -0.35 to 0.51 mm^3), as presented in Figure 6.2.

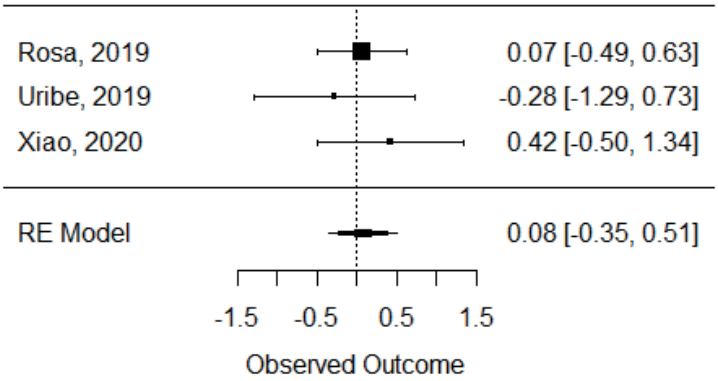

Figure 6.2. Forest plot for the standardized mean difference obtained for bone formation volume analysis synthetic measures reported in the included systematic reviews.

Bone formation percentage analysis

The quantitative analysis of previous meta-analysis synthetic measures was possible considering 4 systematic reviews (Rosa et al. 2019, Wu et al. 2017, Xiao et al. 2020 and Uribe et al. 2019)[42,45-47] and has presented high heterogeneity between studies (I^2 = 93.8%), perhaps due to Wu et al. 2017[45] study which is the only one presenting a statistically significant effect. Despite this, a global standardized mean difference between ICBG and BMP-2 was estimated and found not to be statistically significant (p = 0.184) and was estimated to be 67.92% + 51.05% (95%CI: -32.13 to 167.97%), as presented in Figure 6.3.

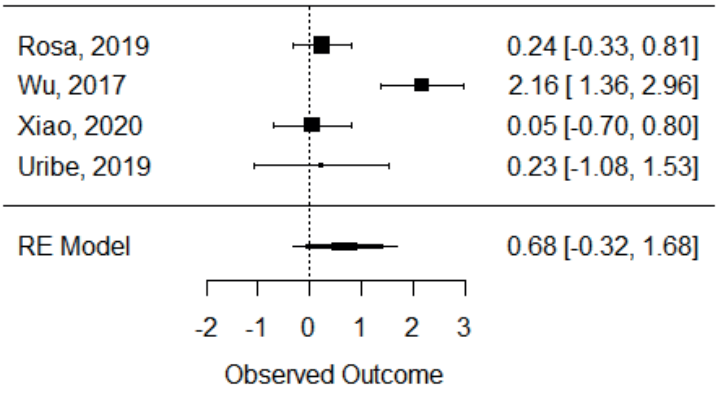

Figure 6.3. Forest plot for the standardized mean difference obtained for bone formation percentage analysis synthetic measures reported in the included systematic reviews.

Bone height

The quantitative analysis of bone height was possible with 3 systematic reviews (Uribe et al. 2019, Xiao et al. 2020 and Scalzone et al. 2019)[42,43,47] and heterogeneity between studies was found to be almost the maximum (I^2 = 99.88%), as Xiao et al. 2020[42] presents a large statistically significant effect when compared to the others. Despite this, a global standardized mean difference between ICBG and BMP-2 was estimated and found not to be statistically significant (p = 0.520) and was estimated to be 5.13% + 9.97% (95%CI: -10.49 to 20.74%), as presented in Figure 6.4.

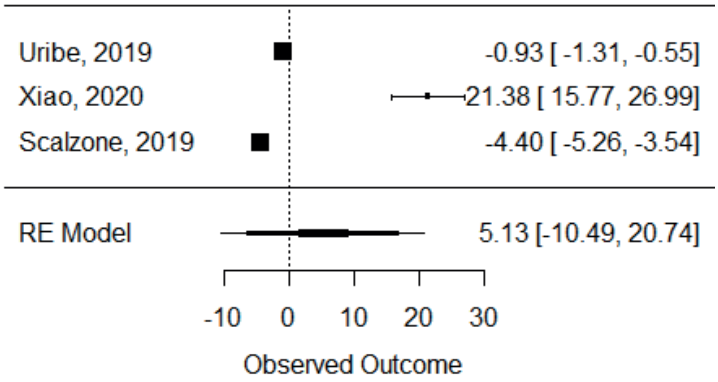

Figure 6.4. Forest plot for the standardized mean difference obtained for bone height synthetic measures reported in the included systematic reviews.

Quality of the evidence

Bone formation volume analysis

The effect size reported by the studies used in the previous meta-analysis do not depend on the precision of the studies (Egger regression b = 0.04; p = 0.931) neither are correlated to the studies variance (Begg-Mazumdar: t_{Kendal} = 0.333; p = 0.999) indicating lack of publication bias concerning the influence of sample size and variability on the mean difference between treatments.

Bone formation percentage analysis

The effect size reported by the studies used in the previous meta-analysis does not depend on the precision of the studies (Egger regression b = -0.08; p = 0.939) neither is correlated to its variance (Begg-Mazumdar test: t_{Kendal} = 0.000; p = 1.000).

Bone height

The effect size reported by the studies used in the previous meta-analysis does not depend on the precision of the studies (Egger regression b = -4.17; p = 0.485), neither is it related to the variance of the effects reported (Begg-Mazumdar test: t_{Kendal} = 0.333; p = 0.999), indicating lack of publication bias.

4. Discussion

This umbrella review aimed to synthesise the current literature regarding bone strategies in cleft patients, evaluating their success or failure based on systematic reviews with/without meta-analyses.

Conventionally, autologous bone graft using cancellous bone is considered the gold-standard technique for bone graft.[14] All studies included that had a comparator group used autologous bone graft as control. The effectiveness of tissue regeneration approaches was also investigated, with human bone morphogenetic protein being the most reported in 6 of the 9 systematic reviews included. BMP-2 is usually delivered in an alloplastic bone graft or scaffold and is an effective inducer for bone and cartilage formation, belonging to the transforming growth factor-beta proteins superfamily. This protocol avoids the limitations of autologous bone grafts, as limited donor supply, donor site morbidity and reduces the patient's surgical stress, which may be related to the lower operative time and hospital stay length, reported by some studies.[48-49] Yet, some adverse effects such as nasal stenosis and localized graft-site oedema still persist.[42,43,50]

Although some of the systematic reviews included indicated that BMP-2 treatment may benefit bone formation compared to autogenous bone graft, this umbrella review reported no significant difference between these two protocols regarding volume, filling, and bone height.[43,47] However, these findings should be interpreted with caution because clinical and methodological heterogeneity can influence the magnitude of the statistical heterogeneity reported. This umbrella identified different heterogeneity factors, namely, the number of participants, type of cleft, the timing of outcome, and intervention design. Thus, the results of the included studies can be affected by this heterogeneity, and, consequently, the results of this umbrella can also be affected. Additionally, some SR results could not be used for the meta-analysis due to different synthesis measures, decreasing the number of included studies which can affect the obtained result. Nevertheless, the quality assessment revealed that only one study included has a high risk of bias, which allows having more confidence in the results of this umbrella. Therefore, both methods can be an option in cleft bone graft treatment.

The timing for the bone graft is a variable that clinicians should consider because it can influence the bone graft prognosis, endangering the support provided for teeth eruption, the continuity of dental arch and the closure of the oronasal fistula.[50] Despite the difference in the selected studies' age groups, the majority performed the bone graft after 8 years of age, which is the ideal timing reported in the literature.[13,51,52] Furthermore, a recent study by Brudnicki et al. reported that bone graft performed before 8 years old might have a limited negative effect on craniofacial morphology.[50]

Regarding the postoperative newly formed bone evaluation, most included studies used computed tomography. Even though this method has several advantages when compared with two-dimensional tools (eg control teeth eruption process into the bone graft and the assessment of the dimensional location of the bone graft), it has some limitations when compared with cone-beam computed tomography (CBCT).[53-56] CBCT scans have a lower radiation dose, minimal scanning time (10–70 s) and allow the clinicians to scan a small region for specific diagnosis with less image artefact.[57] The orofacial cleft patients require a 3D analysis for the correct diagnosis since they present several medical conditions, namely bone graft interventions, impacted teeth or supernumerary teeth. This is the reason why orofacial cleft patients have CBCT indication by the European Academy of Dental and Maxillofacial Radiology.[58] Therefore, further studies should use CBCT as an assessment tool to measure newly formed bone.

Follow-up times are not consensual, even though most of the trials included in the systematic review reported a follow-up of six months. This period is ideal for carrying out radiograph control since the remodeling process with cortical maturation occurs after six months, remaining stable until the 24th month. Regarding the included studies, only Scalzone et al. considered trials with at least six months of follow-up.[43] The remaining systematic reviews' findings should be carefully analyzed since the remodeling process may not be completed.

This umbrella review has several strengths. Firstly, it provides a comprehensive overview of the available systematic reviews published following a registered protocol with transparent methodology. Moreover, the quality of the individual studies included was assessed using an AMSTAR 2 tool.

The findings of this umbrella could be affected by the methodological and clinical heterogeneity among the included studies. Considering the risk of bias evaluated using the AMSTAR-2 tool, the items that presented more studies with low quality were: funding of included studies, discussion for the heterogeneity, risk of bias in the discussion, inclusion criteria and comprehensive search. In future systematic reviews, the authors should state their funding sources, namely by industry, as this may introduce a bias in the results presented. Most of the included studies failed to discuss the heterogeneity and the risk of bias, especially in justifying the inclusion of studies with different methodology and the consequent bias. Therefore, their conclusions must be interpreted with caution. Before starting the systematic review is essential to reduce methodological flaws, bias and duplication risk. Several SR included in this umbrella presented some gaps in the search description and inclusion criteria definition, which potentially increase the risk of publication and reduce the comprehensive nature of the review. Thus, the registration of the protocol is recommended and will enhance the robustness in further research. Although systematic reviews are considered the most reliable evidence, the studies included in each SR also had associated bias. The methodological heterogeneity

includes differences in the trial settings, missing a priori adequate sample size calculation, type of sample included (eg type of cleft, age groups), intervention protocols, bone measurement tools and follow-up times. Other variables may affect the analysis of primary outcomes since they affect bone remodeling, namely, the position of teeth on bone graft, the cleft defect's width, and the volume of grafted bone. The primary outcomes may also be affected by the clinician´s expertise and the research group scientific proficiency. Secondarily, most selected studies were categorized as having a low or moderate overall quality, which may decrease the findings' certainty. Moreover, the included studies can perform an overestimation of the findings' effects due to the inclusion of several publications of a single study or by excluding studies in other languages. Finally, SR without meta-analysis may present a high risk of bias. Therefore, the findings of this umbrella should consider these limitations in the interpretation of results.

Future research should carry out blinded RCTs to control possible sources of bias such as randomization procedure, measurement tools and follow-up times. Moreover, the cost-benefit analysis of these new regenerative strategies in the bone graft is recommended since it plays a crucial role in healthcare systems' decision-making.

5. Conclusions

This umbrella review supports that BMP-2 and autogenous bone graft have similar effectiveness regarding volume, filling, and bone height in oral cleft patients' surgery.

However, the findings should be analysed cautiously due to several research gaps concerning the original studies' methodological quality

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VII

Chapter VII

Platelet-rich fibrin in bone regenerative strategies in orthodontics: a systematic review

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1. Introduction

Regenerative therapy in oro-dental and maxillo-facial defects is challenging because oral cavity has several tissues with distinct cell populations (ectodermal and mesodermal), making the regenerative procedures more complex.[1] Bone and soft tissue regeneration may be indicated for managing defects subsequent from several conditions, such as congenital defects (cleft lip and palate), alveolar bone resorption, periodontal defects (recession coverage and furcation defects), cystic cavities, bone infection (osteomyelitis) and traumatic bone destruction.[1-4] Nowadays, the current clinical approaches have several limitations, namely limited self-renewal capacity and/or limited donor supply, risk of immune response, operative time and costs and donor site morbidity. As a consequence, new biomaterials have been developed to modulate inflammation and enhance the healing process.[5]

Platelets derivatives are increasingly used in regenerative dentistry, particularly in implantology, oral surgery and periodontology. Platelets, 2 – 3 μmm blood corpuscles, are cytoplasm fragments from the megakaryocytes in the bone marrow that then enter the circulation. Following tissue injury, activated platelets have a key role in soft and hard tissue regeneration. Platelet concentrates release a variety of cytokines and growth factors that promote the regenerative capacity of periosteum and improve bone and tissue healing and regeneration. Choukroun et al. reported that the platelet-rich fibrin (PRF) improves tissue repair and regeneration. PRF is prepared from centrifuged autologous blood with no addition of bovine thrombin or anticoagulants.[6] During blood centrifugation two processes occur: 1) blood coagulation; 2) separation of blood elements due to the centrifugation force. Subsequently, three distinct layers are formed: platelet-poor plasma (top); PRF (middle zone); and red blood cells (bottom).[7]

This fibrin matrix contains platelets, leukocytes, growth factors and cytokines such as interleukin (IL)-1β, IL-4 and IL-6, transforming growth factor-beta1 (TGF-β1), platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF).[6-7] These factors can promote the proliferation/differentiation pathway of osteoblasts, endothelial cells, chondrocytes, and various sources of fibroblasts, which can stimulate the regenerative capacity of periosteum and enhance

bone and tissue repair and regeneration.[8] Furthermore, the fibrous structure of PRF acts as a three-dimensional fibrin scaffold for cell migration.[9] Thus, PRF may be used with bone substitutes, which allows wound sealing and hemostasis, improve bone maturation and graft stabilization. Furthermore, PRF membrane can be used for guided bone regeneration.[2]

Tissue regeneration is a new emerging approach in orthodontics because a high percentage of patients need both regeneration and orthodontic treatment. Orthodontic treatment can be performed on children, young adults and adults. All of these patients may need regenerative approaches due to different indications (eg children with cleft lip and palate who need closure of alveolar cleft; older patients who need an orthodontic treatment due to bone defect as a result of tooth loss). Moreover, the application of mechanical force on the teeth affects the periodontal ligament and the alveolar bone, which allows orthodontic tooth movement (OTM).[10] Thus, a change in support structures may interfere with orthodontic success. Therefore, the use of PRF can improve orthodontic treatment result, since it promotes a biological response involving a minimally invasive procedure. Moreover, PRF is completely autologous, requires minimal biochemical handling of blood, provides release of growth factors over time, it is easy to prepare and cost effective.[11] During the last years, clinical applications and effects of PRF in regenerative dentistry have been reviewed, but studies on the application of PRF in orthodontics are sparse. The purpose of this review was to systematically investigate and appraise the quality of the most up to date available evidence from human studies regarding the applications and effects of PRF in orthodontics.

2. Materials and Methods

Protocol

This systematic review was designed and reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines and Cochrane guidelines for systematic reviews.[12-13] The PICO (Population, Intervention, Comparison and Outcome) research question was: "What is the application and effects of platelet-rich fibrin in orthodontic treatment?".

The protocol for this systematic review was registered on PROSPERO (Reference: CRD42020162578).

Eligibility criteria

Table 7.1 describes the PICO research question.

Table 7.1. Research question according to the PICO format

Parameter	Assessment
Population (P)	Orthodontic patients of any sex or age.
Intervention (I)	Participants who underwent treatments approaches with the use of PRF with/without a combined biomaterial.
Comparison (C)	The control group consisted of participants that underwent treatments approaches without PRF.
Outcome(O)	Outcome were: • hard tissue reconstruction of alveolar bone - assessed by volume of the newly formed bone (measured in cubic centimeter or percentage of newly formed bone); • rate of tooth movement - assessed by the change in horizontal linear distance between the mid-marginal ridges of the adjacent teeth (measures in millimeters).

Search Strategy and Study Selection

Four electronic databases (Medline via PubMed, Cochrane Library, Web of Science Core Collection and EMBASE) were searched until 16th December 2019 independently by two reviewers (I.F., F.V.).

To conduct the research, a combination of Medical Subject Headings (MeSH) with relevant free text words was used in each database. Appendix 7.1 summarised the search strategies. The following language filters were applied: English, Portuguese and Spanish. Furthermore, no restrictions of publication date were applied. A manual searching of the references lists of the retrieved full text articles was also conducted.

Articles were screened based on the titles and abstracts according to the eligibility criteria by two independent reviewers, in duplicate. Subsequently, full texts were screened for potential inclusion and disagreements were resolved through mediation with a third reviewer (M.F.).

The following inclusion criteria were considered: (i) randomised controlled trials (RCTs), controlled clinical trials (CCTs) and cohort studies; (ii) studies in humans; (iii) orthodontic patients; (iv) reported hard tissue reconstruction or rate of tooth movement as outcome (v) the study should evaluate the applications and effects of PRF on orthodontics. The exclusion criteria were as follows: (i) non-clinical studies and all other research types (for example, editorials, textbooks, and technical reports); (ii) edentulous patients; (iii) animal studies; (iv) case reports or descriptive studies; (v) repeated publications; (vi) studies with missing data.

Data Extraction

For data extraction, a standard form was developed. The information that was extracted from each article included: field of study, first author and year of publication, study design, aim of study, number of participants in experimental and control group, PRF protocol, results and main conclusions. In the case of uncertainty or discrepancies between the reviewers (I.F., F.V.), a third reviewer was consulted (M.F.).

Risk of Bias

Two reviewers assessed the methodological quality of recruited studies independently. For both RCTs and CCTs, the Cochrane Risk of Bias tool was used.[14] The domains evaluated were: (1) random sequence generation; (2) allocation concealment; (3) blinding of participants and personnel; (4) blinding of outcome assessment; (5) incomplete outcome data; (6) selective reporting; (7) other bias. Risk of bias is detailed in appendix 7.2. The overall risk of bias of individual studies was categorized as low (if all domains were considered as having a low risk of bias), unclear risk (if one or more domains were at unclear risk of bias) and high (if at least one domain was at high risk of bias).

For cohort studies, the qualitative assessment of the selected studies was performed using the Risk of Bias in Non-randomized Studies – of Interventions (ROBINS-I) assessment tool.[15] The domains evaluated were: (1) bias due to confounding; (2) bias in selection of participants into the study; (3) bias in classification of interventions; (4) bias due to deviations from intended intervention; (5) bias due to missing data; (6) bias in measurement of outcomes; (7) bias in selection of the

reported result. This information is summarized in appendix 7.3. The overall risk of bias of individual studies was categorized as low (if all domains were considered as having a low risk of bias), moderate (if low or moderate risk of bias for all domains), serious (if at least one domain was at serious risk of bias), critical (if at least one domain was at critical risk of bias) and no information (if no clear indication that the study is at serious or critical risk of bias and there is a lack of information in one or more key domains of bias).

3. Results

Selection of the studies

A total of 489 studies were identified from electronic databases. After the removal of duplicates using EndNote reference management software (Clarivate Analytics, Philadelphia, Pennsylvania, USA), 426 records were screened based on the titles and abstracts. In the case of uncertainty or discrepancies, the article was included. Subsequently, 25 studies were reviewed and evaluated according to the eligible criteria. The final sample included 9 studies.

The included studies were single centre studies. All included articles were parallel two-arm trials[2,16-21] except the two studies that were split-mouth trial.[22-23]

The identification, screening and eligibility process is summarised in the PRISMA flow chart (Figure 7.1).

The reasons for the excluded records are summarised in appendix 7.4.[24-39]

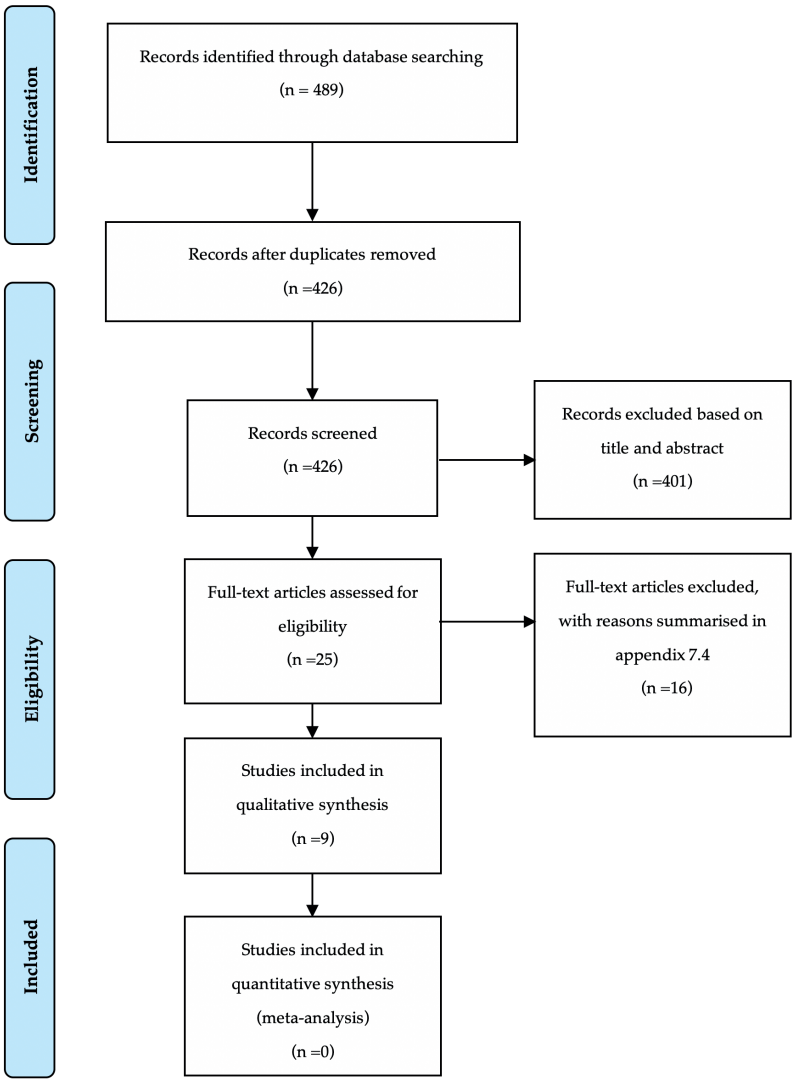

Figure 7.1. PRISMA flow chart

Characteristics of included studies

The general information of included studies is summarised in tables 7.2 and 7.3. The studies involved a total of 175 patients aged 6-51 years, and were recent, undertaken between 2016 and 2019. Six studies[2,16-20] investigated the efficacy of PRF in maxillary alveolar cleft reconstruction. Four of them compared autogenous bone graft from anterior iliac crest with and without PRF. The other two studies[17-18] investigated the combination of PRF with other approachs: 1) allogenic bone material and chin symphysis bone[17]; 2) autologous bone marrow mononuclear cells (BMMNCs) combined with nanohydroxyapatite[18]. In the six studies, the cone beam computer tomography (CBCT) or computed tomography (CT) was used as an assessment tool to measure outcome.

Three articles investigated tooth movement and post-orthodontic stability.[21-23] Two studies evaluated the amount of OTM by decreasing the horizontal linear distance between the mid-marginal ridges of the adjacent teeth.[22-23] Only one study considered clinical parameters and patient feedback to evaluate pain, post-surgical inflammation and infection.[21]

Table 7.2. Characteristics of included studies on alveolar cleft reconstruction.

	Alveolar cleft reconstruction					
Study	Omidkhoda et al.[16]	Movahedian Attar et al.[17]	El-Ahmady et al.[18]	Saruhan et al.[2]	Shawky et al.[19]	Desai et al.[20]
Year	2018	2017	2018	2018	2016	2019
Study design	Parallel-group RCT	Parallel-group RCT	Parallel-group RCT	Parallel-group RCT	Parallel-group RCT	Parallel-group RCT
Aim of study	Efficacy of PRF in the quality and quantity of maxillary alveolar cleft repair.	Efficacy of (i) combination of symphysis bone, allograft and PRF, and (ii) iliac bone graft, in the regeneration of cleft defects.	Use of autologous BMMNCs combined with PRF and nanohydroxyapatite as an effective technique for alveolar cleft repair.	Effect of PRF in alveolar bone grafting using volumetric analysis.	Effect of PRF in the quality and quantity of unilateral maxillary alveolar cleft repair.	Efficacy of PRF for secondary alveolar bone grafting.
Interventions	Autogenous anterior iliac graft with PRF (n=5)	Bone graft from allogenic bone material, chin symphysis bone and L-PRF (n=10)	Autologous BMMNCs combined with nanohydroxyapatite and autologous PRF (n=10)	Autogenous bone graft from anterior iliac crest with PRF (n=17 alveolar cleft segment)	Autogenous bone graft from anterior iliac crest with PRF (n=12)	Autogenous bone graft from anterior iliac crest with PRF (n=20)
Control	Autogenous anterior iliac graft (n=5)	Autogenous bone graft from anterior iliac crest (n=10)	Autogenous bone graft from anterior iliac crest (n=10)	Autogenous bone graft from anterior iliac crest (n=14 alveolar cleft segment)	Autogenous bone graft from anterior iliac crest (n=12)	Autogenous bone graft from anterior iliac crest (n=20)
Sample size (females/males)	10 (4/6)	20 (9/11)	20 (12/8)	31 alveolar cleft segments in 22 patients (13/9)	24 (8/16)	40 (19/21)
Participant age (mean ± SD)	9-12 (11.3 ± 0.83)	8-14 (9.7 ± 1.7)	8-15 (11.50 ± 7.55)	6-28 (17.71 ± 5.4)	9-14 (10.92 ± 2.75)	9-18 (15.29 ± 4.79)
PRF protocol	3000 rpm, 10 minutes	3000 rpm, 10 minutes	3000 rpm, 20 minutes	3000 rpm, 10 minutes	3000 rpm, 10 minutes	2900 rpm, 10 minutes
Outcome assessed	CBCT images (Planmeca, Finland, 2009). Exposure parameters: field of view of 90×100 mm, voxel size of 200 µm, X-ray tube kilovoltage of 88 kVp, and 8 mA.	CBCT Images (Cranex 3D, Sordex, Helsinki, Finland). Exposure parameters: 0.5 mm scan thickness for axial cuts.	Panoramic radiographs and CBCT images. Pain was measured with a numerical scale score reporting pain intensity.	CBCT Images (NewTom FP, Quantitative Radiology, Verona, Italy). Exposure parameters: 0.5 mm scan thickness for axial cuts.	CT scan (Philips Brilliance 32 Slice.Cardiac MDCT, Philips Healthcare, city, Netherlands) of upper jaw. The axial cuts were 0.625 mm thick.	Orthopantomogram, upper occlusal view, and CT scan (KODAK 9000C and KODAK 9000C 3D extra oral Imaging System; Carestream Inc., 2016).
Follow up	3 months. CBCT images: immediate postoperative and 3 months after surgery.	12 months. CBCT images: before surgery and 12 months after. Clinical controls: 1week, 1, 3, 6 and 12 months.	12 months. CBCT images: 6 and 12 months after surgery. Clinical controls: 1 day, 1 and 3 weeks, 6 and 12 months after surgery.	6 months. CBCT images: preoperative and 6 months after surgery. Clinical controls: every week during the first month; every month for the next 5 months.	6 months. CBCT images: preoperative and 6 months after surgery.	9 months. Radiographic assessment: preoperative; immediate, 3, 6 and 9 months postoperative.
Conclusion	PRF group did not have a significant increase in the thickness, height, and density of alveolar bone graft.	Averagely 69.5% of alveolar defects were regenerated with bone in experimental group and 73.8% on control group (p value = 0.156). Chin symphysis bone and allogenic bone material combined with L-PRF was an appropriate graft material.	Experimental group demonstrated 90% of complete alveolar bone union versus 70% in control group. Autologous BMMNCs in combination with autologous PRF and nanohydroxyapatite promote bone regeneration in alveolar clefts defects.	Postoperative newly formed bone volume was better in the experimental group (68.21%) than in control group (64.62%). Although, no statistically significant difference was found.	The mean amount and percentage of newly formed bone volume was higher in the experimental (0.78cm^3; 82.6%) than control group (0.62cm^3; 68.38%). Bone density does not increase, but the difference was not statistically significant.	PRF in combination with autogenous bone results in higher osteogenic effect which increases new bone regeneration and better wound healing.

RCT, randomized controlled trial. PRF, platelet-rich fibrin. SD, standard deviation. CBCT, cone beam computer tomography. L-PRF, leucocyte- and platelet-rich fibrin. BMMNCs, bone marrow mononuclear cells. CT, computed tomography scan. rpm, revolutions per minute.

Table 7.3. Characteristics of included studies on tooth movement and post-orthodontic stability.

	Tooth movement and post-orthodontic stability		
Study	Muñoz et al.[21]	Tehranchi et al.[22]	Nemtoi et al.[23]
Year	2016	2018	2018
Study design	Cohort	Split mouth clinical trial RCT	Split mouth clinical trial CCT
Aim of study	Effect of L-PRF in PAOO concerning post-operative pain, inflammation, infection and post-orthodontic stability.	Effect of LPRF on OTM in extraction cases.	Efficacy of PRF in accelerating bone regeneration and orthodontic tooth movement.
Interventions	Wilcko's modified PAOO technique combined with L-PRF	Extraction socket with LPRF (n=15)	Extraction socket with LPRF (n=20)
Control	---	Extraction socket with secondary healing (n=15)	Extraction socket with secondary healing (n=20)
Sample size (females/males)	11 (8/3)	Thirty extraction sockets in 8 patients (3/5)	Forty extraction sockets in 20 patients (11/9)
Participant age (mean +/- SD)	15-51	12-25 (17.37 ± 12.48)	12-20 (16.43)
PRF Protocol	3000 rpm, 10 minutes	2700 rpm, 12 minutes	2700 rpm, 12 minutes
Outcome assessed	Clinical parameters and patient feedback were used to evaluate pain, post-surgical inflammation and infection.	OTM was measured by comparing the change in horizontal linear distance between the mid-marginal ridges of the adjacent teeth on a regular basis.	CBCT images (PlanmecaPromax 3D Mid, Planmeca OY, Helsinki, Finland). Exposure conditions: 90 kV, 12 mA, and exposure time of 18.3 s. OTM was measured by comparing the change in horizontal linear distance between the mid-marginal ridges of the adjacent teeth on a regular basis.
Follow up	Clinical evaluation: 1, 2, 4, 8 and 10 days post-operative.	16 weeks. OTM measurements: 2, 4, 6, 8, 10, 12, 14 and 16 weeks.	24 weeks. OTM measurements: before placement of PRF; 4, 8, 12, 16, 20 and 24 weeks after placement of PRF.
Conclusion	1) No severe pain; 2) edema resolution begun by day 4 in most patients (72.7%); 3) orthodontic treatment average time was 9.3 months; 4) all cases maintained stability for at least 2 years.	LPRF group: decreased horizontal linear measurement between the mid marginal ridges of teeth (p=0.006). Therefore, L-PRF may accelerate OTM, particularly in extraction cases.	PRF group: decreased horizontal linear measurement between the mid marginal ridges of teeth. Therefore, L-PRF may accelerate OTM, particularly in extraction cases.

RCT, randomized controlled trial. PRF, platelet-rich fibrin. SD, standard deviation. CBCT, cone beam computer tomography. L-PRF, leucocyte- and platelet-rich fibrin. PAOO, periodontally accelerated osteogenic orthodontics. OTM, orthodontic tooth movement. CCT, controlled clinical trial.

Risk of bias

The results of the quality assessment of the RCTs and CCTs studies are summarized in figure 7.2. Three studies were judged as having high risk of bias, mostly due to deviations from the randomization process.[2,22-23] Two trials were judged as low risk of bias.[17,19] The remaining studies were considered as unclear risk due to deviations from the randomization process.[16,18,20] and bias in selection of the reported results.[16,20]

Cohort study was considered as moderate risk of bias due to deviations from the selection of participants into the study, the measurement of outcomes and the selection of the reported results.[21] The remaining domains showed a low risk of bias. The limited number of trials did not allow risk of bias assessment across studies.

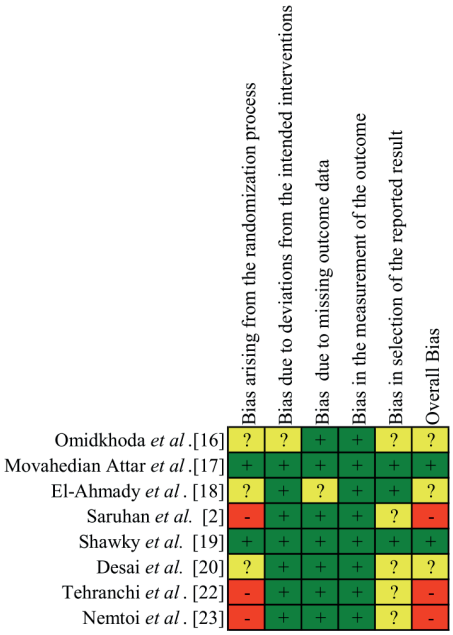

Figure 7.2. Risk of bias of the RCTs and CCTs studies. + low risk of bias. ? unclear risk of bias. – high risk of bias.

Quantitative synthesis of the results

The heterogeneous interventions and treatment performed in the included articles did not allow a quantitative synthesis of the results. Furthermore, the heterogeneity in the design and methodologies precludes the quantitative analysis of results.

Results of included studies

There was a range of different PRF protocol preparation in the included studies. Five studies[2,16-17,19,21] adjusted centrifugation to 3000 rpm for 10 minutes, while others used 3000 rpm for 20 minutes[18], 2900 rpm for 10 minutes[20] and 2700 rpm for 12 minutes[22-23].

The nine studies were grouped under two categories: (1) alveolar cleft reconstruction; (2) tooth movement and post-orthodontic stability.

Alveolar cleft reconstruction

All the works that evaluated alveolar cleft reconstruction comprised a control group in which patients went through maxillary alveolar cleft reconstruction with autogenous anterior iliac crest bone graft.[2,16-19] Four studies compared autogenous anterior iliac graft with or without PRF.[2,16,19-20] Omidkhoda et al. showed that PRF combined with autogenous bone did not have a significant increase in the thickness, height, and density of alveolar bone graft.[16] The other three studies suggested that experimental group had higher amount of newly formed bone.[2,19-20]

Saruhan et al. reported that the mean percentage of newly formed bone was 68.21±10.80% and 64.62±9.49% in experimental and control group respectively.[2] Desai et al. evaluated vertical bone height with 4-point scale (type 1- 0-25% resorption; type 2- 25 to 50%; type 3- 50 to 75%; type 4- 75

to 100%). In the experimental group, 18 patients had grade 1 resorption and 2 patients had grade 2 at 9 months. In control group, 12 patients had grade 1 resorption and 8 patients had grade 2 at 9 months.[20] Shawky et al. verified that the experimental group had higher percentage of newly formed bone (82.60%) compared with control group (68.38%). This study is the only one with statistically significant results assessing the percentage of newly formed bone.[19]

Concerning the mean bone density, experimental group demonstrated lower values than control group with no statistically significant differences. Shawky et al. reported values of 384.03 HU and 360.82 HU, respectively for control and experimental groups, at 6 months follow-up.[19] Omidkhoda et al. also verified that the mean bone density was lower in experimental group (302.83 HU) than on control group (349.58) at 3 months after surgery.[16]

The two studies that included other materials have found that PRF combining to other regenerative materials was an appropriate graft material for reconstruction in alveolar clefts defects.[17,18]

Movahedian et al. evaluated the efficiency of the combination of bone graft from allogenic bone material, chin symphysis bone and L-PRF and verified that the percentages of bone reconstruction were lower in experimental group (69.57 ± 10.13%) than in control group (73.86 ± 6.93%), without statistical differences between the two groups.[17] Otherwise, El-Ahmady et al. showed that 70% of the experimental group (using PRF with autologous BMMNCs and nanohydroxyapatite) presented bone tissue at the cementoenamel junction of the teeth next to the cleft, covering at least 75% of both roots against 30% of the control group, at 12 months follow up.[18]

The most frequent outcome measures were volumetric measurements and percentage ratios such as height, thickness and length. Four studies evaluated augmentation, bone reconstruction and graft ratios by comparing pre and postoperative 3D X-rays.[2,17,19-20] The evaluation of alveolar resorption or residual bone ratio and postoperative follow up was performed using 3D X-rays.[16,18] The follow–up ranged from 3 to 12 months across the studies. During the follow-up period, different outcomes were reported. Two studies did not verified complications (dehiscence, flap necrosis and infection or persistent oro-nasal fistula)[17,19], whereas other two studies identified persistent oro-nasal fistula in 30% of the control group[18] and dehiscence in 4 patients in the experimental group (n = 20) and 8 patients in control group (n = 20)[19]. The other two included studies[2,16] did not report any information.

Tooth movement and post-orthodontic stability

Two trials evaluated the effect of L-PRF or PRF on orthodontic tooth movement. [22-23] Both studies showed that application of these platelets' derivatives in the extraction socket can accelerate orthodontic tooth movement (p=0.006)[22,23], and it has been shown by Tehranchi et al. that there was no statistical difference between teeth in the maxillary and mandibular arch on OTM rate[22].

The study by Muñoz et al. was the only one that evaluated the effects of L-PRF considering inflammation and post-orthodontic stability. They demonstrated that in a high percentage of patients (72.7%) edema resolution was set about day 4, and orthodontic stability was preserved for two or more years post-surgery in all patients.[21]

4. Discussion

Alveolar cleft reconstruction and accelerated orthodontic tooth movement are matters of concern in contemporary orthodontics.[28,40] However, few studies were found assessing application of PRF on orthodontics compared to other dentistry areas.

All included articles in this systematic review studied the advantages of PRF in orthodontics field. Although quantitative report of the findings was not possible, qualitative systematic reviews still improve understanding and having a critical appraisal of research relevant to regenerative orthodontics.

To evaluate the postoperative newly formed bone, 2D or 3D X-ray are required in addition to clinical assessment. Three-dimensional radiological studies had some benefits compared with two-dimensional ones, namely the three-dimensional location of the bone graft and the assessment of teeth eruption process on alveolar graft.[41] Advantages of CBCT over CT include the possibility to scan small regions for specify diagnosis, minimal scanning time (10–70 seconds), low radiation dose and reduced image artefact.[42] In the present study, CBCT scan was used in five trials[2,16-18,23] and CT scan in two trials[19,20]. Due to its high performance, CBCT should be a standard treatment outcome method for assessment of newly formed bone in further studies.[28] According to Thuaksuban et al., the remodelling process with cortical maturation occurs after 6 months, becoming stable until month 24.[43] Therefore, CBCT should be carried out 6 months after the bone graft. Regarding the included studies, Omidkhoda et al. only evaluated a 3 months time-point[16], thus results should be carefully analysed, as the remodelling process may not be completed.

Orthodontic treatment combined with surgical approaches is a common procedure in cleft lip and palate patients. In these patients, the treatment begins at birth and continues into adulthood usually requiring prosthesis in an anterior region or mesial movement of the posterior teeth to space closure of agenesis, mainly of the upper lateral incisor.[44] Alveolar cleft reconstruction with bone graft allows the adequate volume of alveolar bone, which is fundamental for the dental movement in the maxillary aesthetic zone throughout the orthodontic treatment. Thus, the orthodontist can perform more stable and aesthetic treatments. In patients with alveolar cleft, autogenous iliac crest bone is the

gold standard.[45] Autogenous bone graft is osteoconductive, osteoinductive and a source of osteogenic precursor cells.[19] However, new strategies, such as the use of PRF, have been advanced to speed up bone formation, reduce bone resorption and enhance soft tissue healing. PRF is a platelet concentrate without addition of thrombin or anticoagulants.[6] The physiologic polymerization in PRF allows the cytokines and growth factors to be stored and then slowly released, ensuring bioactive levels for a long time-period (up to 28 days).[46] Besides protecting the surgical site, PRF membranes promote soft tissue healing functioning like a matrix to support neoangiogenesis, and migration of stem cells and osteoprogenitor cells into the graft.[6,19] In line with this, PRF decreases bone resorption and hasten wound healing in soft and hard tissues[11], which might contribute to the lower prevalence of complications during the follow-up period observed in the PRF group compared to the control group, reported by El-Ahmady et al.[18] and, also, to the increased new bone regeneration and better wound healing observed by Desai et al.[20]. These results are in line with other stating that PRF increased significantly root coverage.[47,48]

Regarding orthodontic tooth movement, several non-invasive or invasive techniques have been proposed for accelerating this process. Most non-invasive techniques need more studies to prove their clinical effectiveness.[49] Invasive techniques appear to be more effective in promoting orthodontic tooth movement. The bone injury associated to the surgical procedure triggers a tissue reaction that enhances normal molecular and cellular events involved in tissue healing.[50] Somehow, the application of PRF mimics the surgical-induced healing capabilities, also inducing tissue regeneration. Being a physiologic polymerized fibrin matrix, PRF incorporates platelets, leucocytes, bioactive molecules and trapped circulating stem cells and progenitors to promote local tissue healing.[6] The two trials included in the present systematic review showed that the use of PRF or L-PRF in the extraction socket could accelerate OTM ($p = 0.006$), specifically in the beginning of orthodontic treatment (alignment and leveling).[22,23] These results are in line with previous ones reporting that the application of several bioactive grafts can increase the bone maturation without interfering with the natural healing process.[51] However, cytokines and growth factors levels are maintained for a long time-period (up to 28 days). Liou demonstrated that the clinical effect of application of platelets derivatives could last 5 to 6 months with the faster rate of orthodontic movement in the 2nd to 4th month.[52] Although no conclusion of the potential effect of PRF on this process could be drawn based on these two trials, there was a trend that PRF has the ability to increase tooth movement. Thus, the application of PRF may shorten the orthodontic treatment time reducing associated costs, which nowadays is a concern in orthodontic patients, specifically in adults and patients with longer treatments such as those needing tooth extractions. Nevertheless, PRF has some disadvantages, namely the limited volume that can be produced and used; also, tissue banks are impracticable, as it is specific to the doner and can not be used as an allogenic graft tissue.[6]

Limitations in this review

The methodological and clinical heterogeneity among studies only allowed to qualitatively account the findings of this systematic review. Newly formed bone measurement tools were inconsistent across studies. Furthermore, most of the selected studies were classified as having a high or unclear risk of bias, which may decrease the certainty of the results. The heterogeneity

of studies can be justified by the methodological differences across the studies, such as sample sizes, intervention protocols and follow-up times. Furthermore, several factors can affect local bone remodeling, namely age at surgery, width of the cleft defect, volume of grafted bone and position of teeth on bone graft.

The authors recognize that the expertise of the clinician and support team, as well as the scientific proficiency of the all research group, influence the outcome evaluation. Some of the selected studies did not assess this factor, which should be considered when figure out the results of this review.

Recommendations for future research

Given the inconsistent results presented in the limited literature, it is recommended to perform further research with standardized methodologies, larger sample size and longer follow-up periods are required to evaluate the applications and effects of PRF in orthodontics field. Possible sources of bias should be controlled such as randomization procedure, PRF protocols preparations, measurement tools of newly formed bone and follow-up periods. Further studies should also investigate the cost-benefit analysis of using PRF in orthodontics for patients and clinicians.

5. Conclusions

Despite the limitations in the included studies, this systematic review suggested that PRF can improve alveolar cleft reconstruction. Concerning orthodontic tooth movement, the results highlight the positive effects of PRF, since it may shorten the orthodontic treatment time reducing associated costs.

Further high-quality randomised controlled trials with identical methodologies, larger sample size and longer follow-up periods are required.

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Chapter VIII

VIII

The effect of L-PRF membranes on bone healing in an organotypic chick femur model

1. Introduction

The bone loss can lead to changes in support, form and loss of metabolic functions. Alveolar bone regeneration is often required to manage bone defects, which can occur due to several factors such as congenital defects, alveolar bone resorption, trauma, neoplasm resection and bone infection.[1-2]

Currently, various forms of bone graft are accessible (autogenous bone, allograft, and bone graft substitutes) with different properties of osteogenesis, osteoinduction and osteoconduction.[3] Autologous bone graft is considered the gold standard up to now due to the osteoconductive and osteoinductive properties combined with histocompatibility. Despite these advantages, it also presents several limitations such as donor site morbidity, limited donor supply and operative time. Therefore, other forms of bone graft have been proposed but they also present limitations (eg allograft may be the vehicle of transmission of viruses). Moreover, bone grafts alone require a long healing process, which may lead to some rate of relapse (bone resorption can reach up to 40%), increasing the need for reintervention.[1,4] Thus, over the last decade, several natural or synthetic growth factors have been proposed to prevent inflammation and increase bone healing.[5-6]

Platelet derivates are a promising source for autologous cell therapy, especially due to the ability to release cytokines and growth factors, improving soft and hard tissue healing and regeneration. The leukocyte and platelet-rich fibrin (L-PRF) is a second-generation platelet concentrate prepared by centrifugation procedure without bovine thrombin or anticoagulants, forming three distinct layers: platelet-poor plasma; L-PRF in the middle zone; and red blood cells at the end of the tube.[7-8] This concentrate of platelets contains some factors like vascular endothelial growth factor (VEGF), transforming growth factor-beta1 (TGF-β1), platelet-derived growth factor (PDGF) and interleukin (IL)-1β, IL-4 and IL-6 that promote the proliferation and differentiation of fibroblasts, endothelial cells, osteoblasts and chondrocytes inducing tissue regeneration.[7-9] The architecture of L-PRF creates a supportive scaffold for cell migration and angiogenesis, improving the stabilization of bone graft.[10] The rigidity of the fibrin membrane should be considered since it influences the capillary formation by endothelial cells in response to stimulation of the fibroblast growth factor-basic and VEGF. Fibrin

also stimulates the expression of αvβ3 integrin, which will promote the linking between cells, fibrin, vitronectin and fibronectin. So, L-PRF has a crucial role in blood clot formation but also as a reservoir of medular stem cells, allowing their conversion toward the osteoblast cells.[7] Thanasrisuebwong et al. reported that platelet rich fibrin with bone substitute materials works as a signaling protein reservoir for osteoinduction and permits a superior union between bone graft particles, allowing better clinical handling.[11] Likewise, the relative ease of preparation, cost effectiveness and non-immunogenicity allows its application in bone regeneration.[12] Additionally, the presence of leukocytes might prevent infection and help regulate the inflammation process.[13]

Despite the promising characteristics of L-PRF in reducing bone resorption and accelerating soft and hard tissue healing, previous studies have shown contradictory results.[14-16] Al-Mahdi et al. reported that platelet-rich fibrin (PRF) mixed with autogenous bone graft increased bone density after 6 months and reduced postoperative bone resorption significantly.[6] Conversely, Omidkhoda et al. showed no significance differences on the density and height of maxillary alveolar cleft reconstruction.[16] These distinct findings may be due to the lack of standardization across the studies regarding the protocol of preparation, outcome measure methods and follow-up times. On the other hand, it must be pointed out that the current evidence results from in vitro, in vivo and clinical studies with small sample size.[6,17-18]

The ex vivo culture model allows the manipulation of cells and tissues in a spatial arrangement comparable to in vivo models, avoiding the systemic influences. Moreover, this model is more cost-effective and avoids ethical issues present in animal experimentation. The chick embryo model is an established model system for evaluating tissue engineering strategies, particularly bone for several reasons: high response to external stimuli; no immune system in early stages; measures tissue responses over a defined time-frame; and, cost-effectiveness.[19-20] Therefore, this study aims to use the chick embryo model to evaluate the effects of the L-PRF on bone regeneration, in order to investigate whether this may be a viable alternative method to further studies. Additionally, the effect of the direct or indirect contact with the L-PRF membrane on the bone regeneration will be investigated.

2. Materials and Methods

L-PRF membrane preparation and morphologic characterization

Peripheral blood samples were collected into four sterile vacutainer tubes (9 ml) without bovine thrombin or anticoagulants from a healthy human volunteer, after written informed consent (Ethics Committee of Faculty of Medicine of University of Coimbra approval, reference CE-049/2019) (Appendix 8.1). The samples were immediately placed symmetrically into the centrifuge device (IntraSpin™, Intra-Lock), being subjected to the centrifugation process (2700 rpm for 12 minutes) (Figure 8.1A).

Then, the fibrin clots were separated from the red blood cells and placed in Xpression™ box for compression by gravity (Figure 8.1B). Finally, clots were carefully separated using scalpel blade into four sections with approximately 3 mm of wide and 1 mm of thickness: two sections with red cells residues (Figure 8.1C*) and two sections without red cells (Figure 8.1C#).

L-PRF membrane was observed by scanning electron microscopy (SEM). For this, the membrane was fixed (1.5% glutaraldehyde, 30 minutes), dehydrated in graded alcohols, critical-point dried, sputter-coated with gold and analyzed in a scanning electron microscope equipped with X-ray energy dispersive spectroscopy (EDS) microanalysis capability (Quanta 400FEG ESEM/EDAX Genesis X4M).

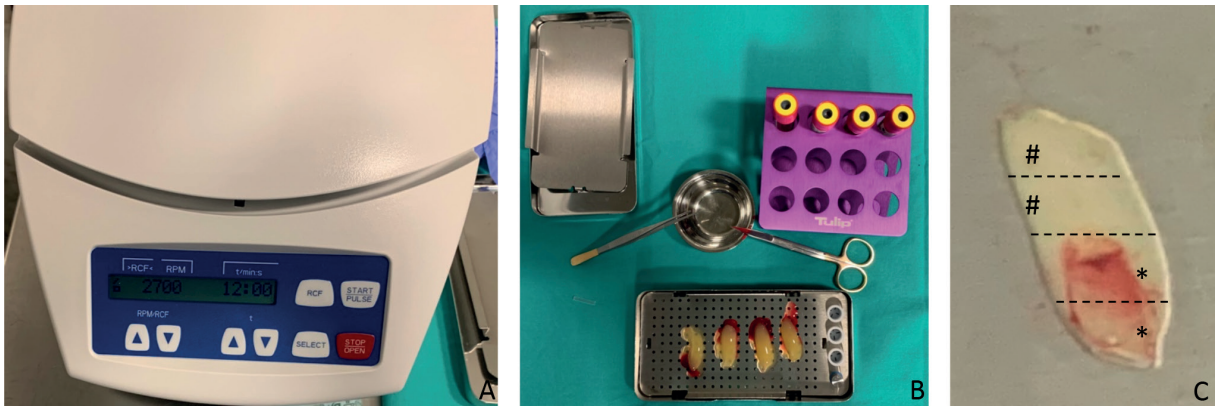

Figure 8.1. Step by step for the preparation of L-PRF: A) centrifugation at 2700 rpm for 12 minutes; B) designed kit (Xpression™ box) to compress L-PRF clots; C) schematic presentation of the cuts made in the L-PRF membrane.

Preparation of segmental chick femoral

For 11 days the chick eggs (Gallus domesticus) were saved in an Octagon 40 ECO rotating egg incubator (Brinsea, UK), with the following conditions: 37.5 °C and 50% humidity. After that, the embryos were euthanized and the muscle and soft tissue of femurs were carefully removed. Subsequently, whole femurs were washed in saline solution and put into a Netwell™ Insert (440 µm mesh size polyester membrane, 30 mm diameter, Corning) in six-well–plates (Costar® 6-well Clear TC-treated Multiple Well Plates, Corning reference 3516) according to the groups:

A. Femur without bone defect and with the application of the L-PRF membrane on top (direct contact) (n=6).
B. Femur without bone defect and with the application of the L-PRF membrane under the Netwell™ insert (indirect contact) (n=6).
C. Femur without bone defect and without the application of the L-PRF membrane as control of the group A and B (n=6).

The basal culture medium (1 mL/well) consisted in Minimum Essential Medium Eagle (α-MEM) with ascorbic acid 2-phosphate (50 µg/mL), penicillin (100 U/mL) and streptomycin (100 µg/mL). Figure 8.2 illustrates the preparation of segmental chick femoral and the placement of L-PRF membranes.

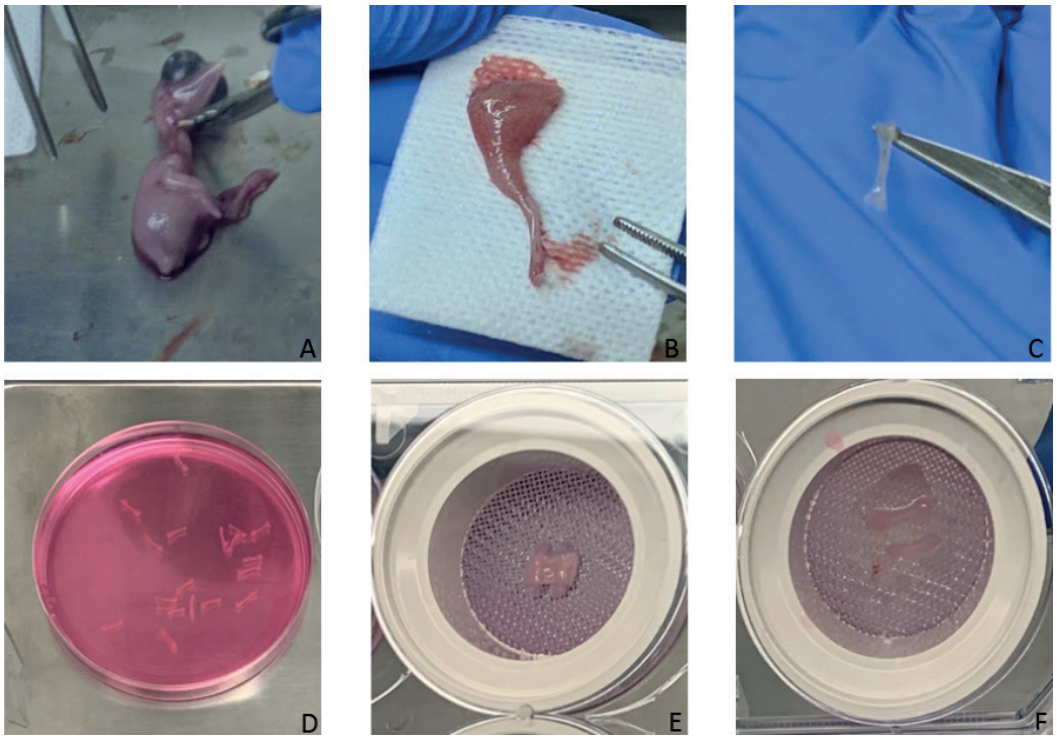

Figure 8.2. Preparation of chick femoral: A) chick embryo; B) chick femur; C) chick femur after dissection; D) femurs in culture media; E) femur of group A; F) femur of group B.

Post-operative monitoring

Femurs were kept for 11 days in basal culture medium at the liquid/gas interface humidified atmosphere of 5% CO_2 in air, and 37°C. Culture media was changed daily. Figure 8.3 shows the cultured femurs at 4- and 7-days post-operative.

At day 11, cultured femurs were washed in phosphate buffered saline, fixed and processed for histological analysis and microtomographic (μCT), using standardized conditions.

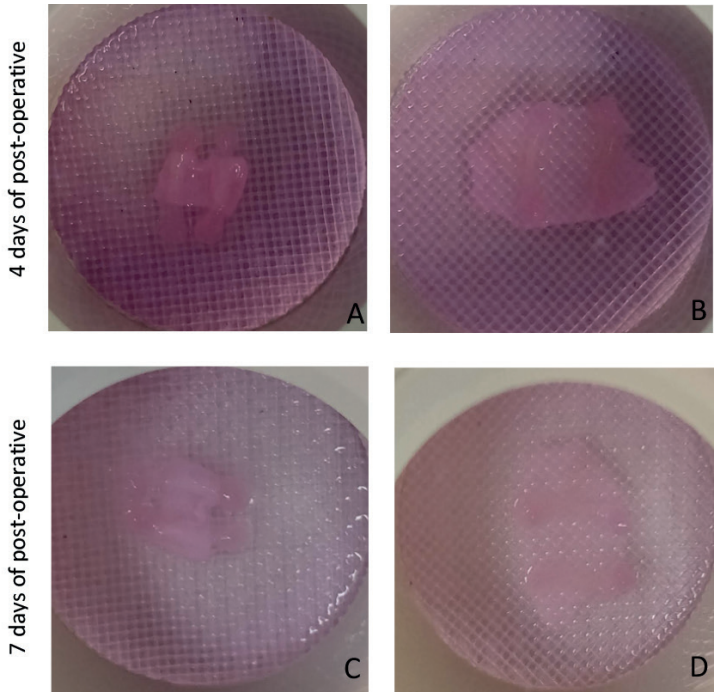

Figure 8.3. Post-operative monitoring: A) femur of group A at 4 days; B) femur of group B at 4 days; C) femur of group A at 7 days; D) femur of group B at 7 days.

Microcomputed tomography (μCT)

Femurs were fixated and stored in a 70% ethanol solution at room temperature.

Micro-CT analyses were performed using a SkyScan 1276 scanning system for μCT (Bruker micro-CT, Kontich, Belgium) (n=4) with the following parameters: 4.5 μm pixel size, 40 kV X-ray voltage, source current 100 μA and an exposure time of 800 ms. The sample containers (1.5 ml Eppendorf tubes) were imaged using a detector assembly over a 360° sample rotation.

The reconstruction and the correction of beam hardening, ring artifacts, smoothening and misalignment parameters was performed by NRecon software v.1.7.4.2. CTAnalyser v.1.17.7.2 (CTAn, Bruker micro-CT, Kontich, Belgium) was used to select a volume of interest (VOI) of 2 mm in the proximal and distal directions, starting at mid-diaphysis and with a total of 900 layers and calculate the following parameters: bone volume (BV), bone surface (BS) and bone mineral density (BMD). The image parameters used were: 40% bean hardening, 11 ring reduction, 1 smoothening and dynamic scale with 0-0.18 range. Three-dimensional images were taken using CTVox software (Brucker, Kontich, Belgium, version 3.3.0).

Statistical analysis

All measurements were performed using the Statistical Package for the Social Sciences, version 26.0 for Windows (SPSS Inc., Chicago, Illinois, USA). A significance level of 0.05 was considered. Descriptive statistics were obtained using mean and standard deviation values. Shapiro-Wilk normality test was performed for each variable, followed by One-Way ANOVA and Tukey's multiple comparisons tests for datasets that presented a normal distribution or Kruskal-Wallis test for non-parametric datasets.

3. Results

SEM images (Figure 8.4) showed that the L-PRF membrane presented a porous morphology resulting from the fibrin strands network. The red portion of the membrane contains abundant clusters of red blood cells and platelets, compared to the yellow portion. Some platelets were also seen in this part of the membrane. High magnification images showed that attached platelets displayed spreading cytoplasm extensions suggesting an activated state.

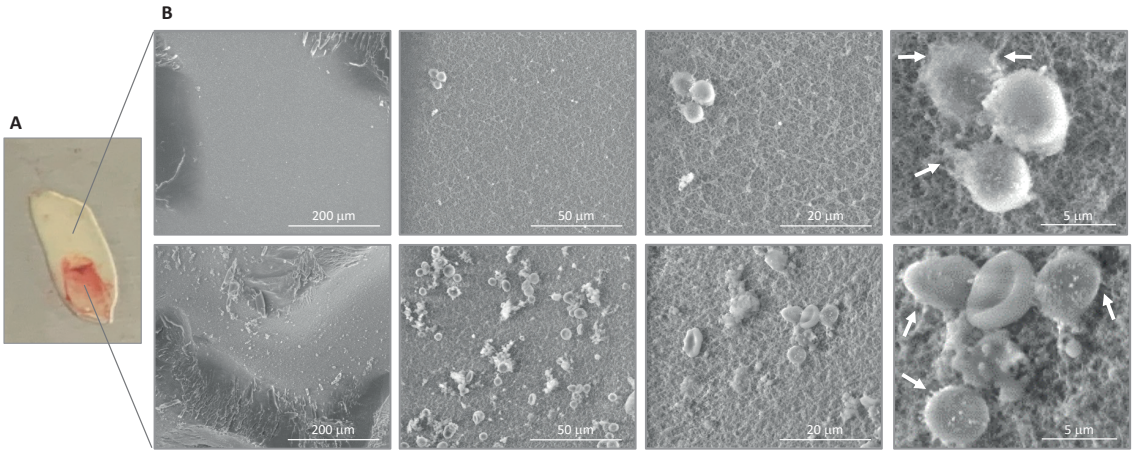

Figure 8.4. Macroscopic appearance (A) and SEM images (B) of the L-PRF membrane. High magnification images showed the porous morphology of the fibrin strands network. The red portion of the membrane contains a high number of erythrocytes and platelets. Attached platelets showed abundant thin cytoplasm extensions suggesting an activated state (arrows).

The osteogenic effects of the L-PRF were assessed through an ex vivo D11 chick femur model. Figure 8.5. A displays the quantitative differences of mineral content among groups – control presented the lowest values for bone volume, bone surface and bone mineral density. Besides, the indirect contact group presented a slight increase in mineral content in comparison to control. However, the direct contact group presented a dramatic increase in mineral content in comparison to other groups, attaining statistical significance regarding bone volume, bone surface and volume ratio (BS/BV). Results suggest that, in the indirect bone/membrane interaction, the growth factors released from

the L-PRF to the medium have beneficial, but limited effects on bone growth. Otherwise, the physical presence of the membrane over the bone (direct contact) unleashes the full potential of the L-PRF effects on bone growth enhancement. Corroborating the quantitative findings, figure 8.5B displays the differences among the groups. It is noticeable that control presented the smallest mineralized area, as well as a dimmer brightness of the diaphyseal region. Comparing the experimental groups, the direct group presents a taller and wider mineral layer at the diaphysis area.

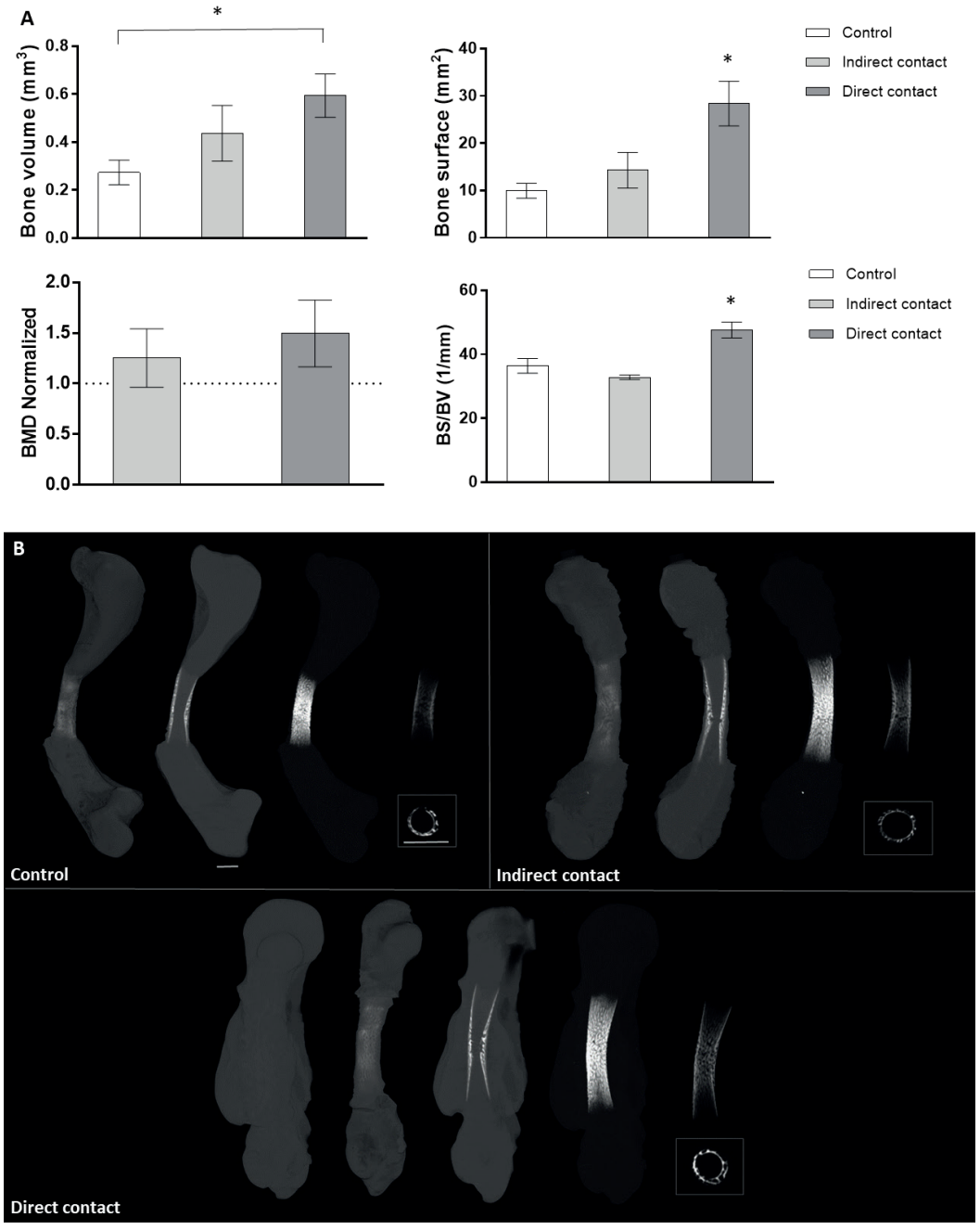

Figure 8.5. Microcomputed tomography analysis of D11 chick femurs following 11-day culture in the presence or in the absence (control) of L-PRF membranes. A – Quantitative morphometric parameters of the samples (BV, BS, BMD and BS/BV). BMD was normalized by the control values, which correspond to the line in the graph. * Statistically significant, $p < 0.05$. B - Three-dimensional representative images of samples (whole femur, sagittal section and maximum intensity projection of the whole femur, sagital/cross-section of the central diaphysis region, respectively). The L-PRF membrane over the bone and the bone isolated from the L-PRF are illustrated in the direct contact samples. Scale 500 µm.

Further, in order to determine if the increased mineral content observed in the direct contact group was restricted to the diaphysis region, the bone was digitally isolated from the L-PRF and then, compared with the complete sample (bone with L-PRF). As observed in Figure 8.6A, the isolated bone presented a minimal decrease in mineral content, suggesting that the most part of the gains in mineral content induced by the L-PRF was, in fact, at the diaphysis region. However, these findings also suggest that a small portion of the mineral content was detached from the bone. To confirm it, the L-PRF membranes were visually scanned and some minor bone sequestration (or isolated bone nodules) were found scattered over the L-PRF membranes (Figure 8.6B).

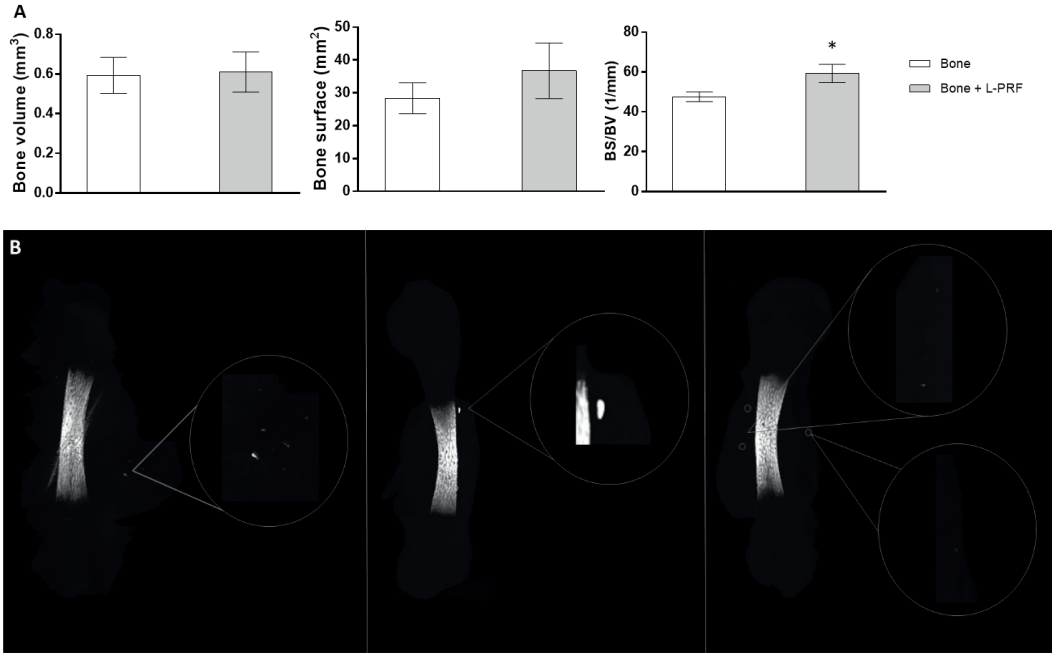

Figure 8.6. Microcomputed tomography analysis within the L-PRF direct contact experimental group. The bone was isolated from the L-PRF in the CTAn' ROI selection and compared with the complete sample (L-PRF over the bone).
A – Quantitative mineral differences of the samples (BV, BS and BS/BV). * Statistically significant, $p < 0.05$.
B – Representative images of bone sequestra (isolated nodules) found across the L-PRF membrane, accounting for the slight increase of quantitative' mineral detection of the complete samples.

4. Discussion

This study aimed to investigate whether the chick embryo model may be a viable alternative method to further studies, as well as the effect of the direct or indirect contact with the L-PRF membrane. It was found that direct contact group had the highest rate in mineral content compared to other groups.

Regarding morphologic characteristics of the L-PRF membrane, the red portion had a higher number of platelets than the yellow portion. Indeed, several studies suggested that the red portion had a more positive effect on bone regeneration than the yellow portion.[11,23] Ehrenfest et al. found that the red portion included a higher number of growth factors and cytokines, while the yellow portion allowed delivery of a fibrin gel serving as a structural support material.[23] More recently, Thanasrisuebwong et al. reported that the red portion had a greater effect on cell proliferation and cell migration, but less fibrin scaffold. This finding may be explained by the fact that the red portion contains more platelets and cells, which will provide a release of more VEGF and, consequently, more cell proliferation and migration. Additionally, these authors also found that the yellow portion stimulated osteogenic differentiation of periodontal ligament stem cells earlier.[11] These findings highlight that the knowledge of the L-PRF architecture is essential in order to make reasonable use in different clinical situations.

The success of bone regeneration depends on two main factors: a structural support material that allows neovascularization and cell recruitment as well as a matrix that deliver morphogenetic, regulatory and growth factors.[24] L-PRF is a reservoir of platelets, so it may have a crucial role in bone regeneration due to several factors, namely: fibroblast growth factor induces preosteoblasts differentiation and proliferation, VEGF enhances angiogenesis and a mitogenic activity and synergy between PDGF with TGF-β1, contributing to the proliferation of cells (marrow stem cells, fibroblasts, pre-osteoblasts) and osteoclasts activity.[25] This study showed that L-PRF groups presented higher values on mineral content for bone volume, bone surface and bone mineral density than the control group. This result is in accordance with the study of Cortellini et al. that showed a significant horizontal ridge augmentation obtained through the L-PRF block.[24]

The direct interaction between platelet-rich fibrin and osseous cells on the healing process is insufficiently documented, presenting contradictory results.[14-16] The distinct results between studies may be caused by the different protocols used regarding L-PRF preparation or surgical procedure. Moreover, Ehrenfest et al. reported that L-PRF differentiates according to the surrounding tissues and cannot survive by itself.[23] Therefore, it is expected that the position of L-PRF membrane will also interfere with the results obtained, since it may lead to less input from growth factors. This study verified this assumption because the physical contact of L-PRF over the bone showed the best result on bone growth enhancement.

Furthermore, the osteogenic response of the direct contact group occurred mostly in the diaphysis region but minor bone sequestration over the L-PRF membrane was also found. This was an unexpected result because Knapen et al. reported that connective cells could be seen in the osteotomy region after the application of L-PRF, but did not extend into the L-PRF membrane.[26]

Previous studies faced great difficulties on PRF research in animal models due to several reasons, namely limited blood volume, variations in experimental design as well as differences in biology and physiology. This ex-vivo model encountered several limitations, namely the combination of L-PRF human samples with chick femur. However, as this study used femurs in early development (embryonic day 11), it did not have yet an immune system.[19] Additionally, L-PRF is used locally and not systematically, which reduce the risk of immunologic reactions and toxic risks. The second limitation is that bone morphometric measurements only assess functionality of the bone indirectly and results are only sensitive to the region of interest.

Despite these limitations, this study presents a viable model for assessing the effects of the L-PRF on bone regeneration. The present model is more cost-effective and avoids several disadvantages of in vivo models, namely bias in data obtained due to systematic influences, large numbers of animals and ethical issues present in animal experimentation. Although, further studies should verify the potential of clinical translation. The association effect of L-PRF and bone graft (autogenous bone, allograft, and bone graft substitutes) on the bone healing defects as well as the potential effect of L-PRF on bone regeneration in systemic diseases, like osteoporosis and diabetes should be investigated.

5. Conclusion

The ex-vivo chick femur is a viable model for assessing the effects of the L-PRF on bone regeneration. L-PRF groups presented higher values on mineral content for bone volume, bone surface and bone mineral density than the control group.

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Conclusions

Conclusions

Key findings in Chapter II

- Regarding statistically significant parental related factors, the probability of having a cleft child decreases for each maternal year increase (OR=0.903) and increases for each body mass index unit (kg/m^2) increase (OR=1.14).
- On the child data birth, for each mass unit (kg) increase, the probability of having a cleft child decrease (OR=0.435). No relation was found regarding length and head circumference.

Key findings in Chapter III

- The achievement of a consensus with regard to cleft care across different centers in Europe remains suboptimal.
- Centralized care was the preferred system and the majority of the countries have an association for cleft patients and professionals (53.6%).
- The largest percentage of patients was seen in the university hospital environment (Fisher's exact test, $p < 0.001$).
- Of the 69 completed surveys, 50 different treatment protocols were described.
- The majority of responders (98.6%) reported that an orthodontist was involved in cleft treatment, and 56.5% of them spend 76-100% of their time treating these patients.

Key findings in Chapter IV

- Rapid maxillary expansion was the preferred expansion protocol (45%).
- Distraction osteogenesis was the most reported alternative treatment to secondary bone grafts (19%), with private practitioners being less likely to perform these treatments (Fischer's exact test, $p = 0.001$).
- Orthodontic services offered were similar in the various locations of provision (hospital and/or university, private).
- Compromised oral hygiene (77%) was the most reported orthodontic complication.
- National Health Services support the majority of cleft orthodontic care (67%) in Europe.

Key findings in Chapter V

- No significant difference was found between groups in OHIP-14 but FIS score revealed a significant difference between the two groups evaluated ($p < 0.001$).
- Only the social limitation in OHIP-14 score revealed a significant difference ($p = 0.001$).
- Regarding FIS score, the most affected dimensions were: family activities ($p < 0.001$), parental emotions ($p = 0.001$) and family conflict ($p = 0.011$).
- Age and sex do not affect the perception of OHIP-14 score.
- Parents of cleft children had a poorer OHRQoL compared to what was perceived by their children and parents of non-cleft children.

Key findings in Chapter VI

- This umbrella review supports that BMP-2 and autogenous bone graft have similar effectiveness regarding volume, height and bone filling in oral cleft patients' surgery. This approach is the most reported in 6 of the 9 systematic reviews included in this umbrella.
- The new approaches avoid the limitations of autologous bone grafts, namely limited donor supply, donor site morbidity and reduces the patients' surgical stress, which may be related to the lower operative time and hospital stay length.

Key findings in Chapter VII

- This systematic review suggested that platelet rich fibrin can improve alveolar cleft reconstruction. This membrane protects the surgical site and promotes soft tissue healing, functioning as a matrix to support neoangiogenesis, and migration of stem cells and osteoprogenitor cells into the graft.
- Concerning orthodontic tooth movement, the results highlight the positive effects of PRF, since it may shorten the orthodontic treatment time reducing associated costs.

Key findings in Chapter VIII

- The ex-vivo chick femur is a viable model for assessing the effects of the L-PRF on bone regeneration.
- L-PRF groups presented higher values on mineral content for bone volume, bone surface and bone mineral density than the control group.

Clinical implications:

Healthcare systems have great problems in affording treatments for cleft lip and palate. Two strategies can be used to reduce costs and improve the quality of healthcare: prevention and the increase of the effectiveness of treatment. Many countries throughout Europe have undergone a significant improvement in surgical and orthodontic healthcare but evidence in cleft treatment is still sparse since the results of current studies are heterogeneous due to several factors such as heterogeneous sample, different treatment protocol and inadequate follow-up.

This investigation opens new doors to regenerative strategies. On one hand secondary bone graft with BMP-2 demonstrated to have great efficacy in bone volume, bone filling, and bone height, as well as in avoiding limited donor supply, donor site morbidity and reducing patient's surgical stress. Simultaneously, platelet rich fibrin can improve alveolar cleft reconstruction by releasing growth factors.

The next reasonable step for prevention research might be the observational studies, which allow the incorporation of their findings into health promotion programs directed at women during reproductive age. Regarding surgical and orthodontic treatment, prospective registries will accelerate the critical appraisal. It would allow the establishment of practice guidelines based on evidence, thus improving surgical and orthodontic outcomes and reducing the burden of care. This will result in a reduced overall cost to the patient and society. Furthermore, the results of this thesis open up new perspectives to the study of 3D-printed scaffolds and substitute materials with or without autogenous bone.

Supplementary files

Appendix 2.1. Ethics Committee (Reference: CE-072/2020)

1 2 9 0 FACULDADE DE MEDICINA UNIVERSIDADE D COIMBRA

COMISSÃO DE ÉTICA DA FMUC

Of. Refª **072-CE-2020**

Data **27/07/2020**

C/C aos Exmos. Senhores

Investigadores e co-investigadores

Exmo. Senhor

Prof. Doutor Carlos Robalo Cordeiro

Director da Faculdade de Medicina de

Universidade de Coimbra

Assunto: Pedido de parecer à Comissão de Ética - Projeto de Investigação autónomo (refª CE-072/2020).

Investigador(a) Principal: Inês Alexandre Neves Francisco

Co-Investigador(es): Francisco Fernandes do Vale e Maria Helena Fernandes

Título do Projeto: ***"Fatores etiológicos implicados no aparecimento da fenda lábio-palatina"*.**

A Comissão de Ética da Faculdade de Medicina, após análise do projeto de investigação supra identificado, decidiu emitir o parecer que a seguir se transcreve:

"Parecer favorável".

Queira aceitar os meus melhores cumprimentos.

O Presidente,

Prof. Doutor João Manuel Pedroso de Lima

HC

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Appendix 2.2. Questionnaire regarding parental risk factors and birth data

1 2 9 0 FACULDADE DE MEDICINA UNIVERSIDADE D COIMBRA

É convidado(a) a participar voluntariamente neste estudo, onde lhe será solicitado o preenchimento de um questionário. Os dados recolhidos são confidenciais e o seu uso é exclusivo para o presente estudo. Este estudo tem como objetivo conhecer os fatores etiológicos da fenda lábio palatina. A sua participação poderá contribuir para melhorar a prestação de cuidados de saúde, nomeadamente a nível do aconselhamento pré-natal.
O preenchimento do questionário terá uma duração máxima de 5 minutos.

1. Data de preenchimento do questionário: ___/___/___
2. Iniciais do nome: _____
3. Sexo do seu filho(a): Feminino__ Masculino__
4. Data de nascimento do seu filho(a): ___/___/___
5. O seu filho(a) tem fenda labial e/ou palatina: Sim ___ Não ___
    a. Se sim, qual?
        i. Fenda bilateral lábio palatina ___
        ii. Fenda unilateral lábio palatina ___
        iii. Fenda Palatina ___
        iv. Fenda Labial ___
6. Data de nascimento da mãe da criança: ___/___/___
7. Data de nascimento do pai da criança: ___/___/___
8. Exposição a fatores teratogénicos durante a gestação? Sim ___ Não ___

    Álcool ___ Tabaco___

    Trimestre: 1° ___ 2° ___ 3° ___

    Mãe___ Pai ___
9. Exposição a outros fatores:

    Medicação (por exemplo, ácido fólico, ferro, iodo):

    _____

    Doenças durante a gravidez (por exemplo, diabetes, hipertensão):

    _____

    Contato com o fumo das lareiras durante a gravidez: Sim ___ Não ___

10. Índice de massa corporal da mãe no início da gravidez: Peso _____ Altura: _____
11. Meio ambiente durante gravidez: Urbano _____ Rural _____
12. Dados ao nascimento da criança:

    Peso _____

    Comprimento _____

    Perímetro cefálico _____

Appendix 3.1. Survey questions and a list of possible answers

1290 FACULDADE DE MEDICINA UNIVERSIDADE D COIMBRA

Question	Answer Options (if applicable)
In what country do you practice?	
What is your specialty (orthodontics, maxillofacial surgery, other)?	
How many years of professional experience do you have?	
How many years does your centre/office collaborate in CLP treatment?	
Please select where your centre/office is integrated:	Hospital environment; University environment; Private practice; University hospital environment;
What is the preferred reference system for CLP patients in your country?	Centralization of services; Local services; Both; Not sure/None of the previous answers.
How far from your centre/office are the majority of your CLP patients?	0-50 km; 51-100 km; 101-150 km; > 150 km.
Please tick the approximate percentage of your patient population in each diagnosis:	Cleft lip Cleft palate Unilateral cleft lip and palate Bilateral cleft lip and palate
Select your centre/office's CLP treatment protocol in chronological order.	Pre-surgical orthopaedics Lip adhesion Lip closure Gingivoperiosteoplasty Soft palate closure Hard palate closure Orthophony Velopharyngoplasty Alveolar bone grafting Dentofacial orthopaedics Orthognathic surgery Lip/Nose Revision
Select the medical specialties involved in CLP treatment at your department/office?	Otorhinolaryngology Maxillofacial surgery Plastic surgery Neurosurgery Orthodontics Paediatric surgery Phoniatrics/ Speech therapy Dentistry Geneticist Child and adolescent psychiatry Clinical nurse specialist
Are there national CLP associations in your country?	Yes, only for professionals; Yes, only for parents; Yes, for both; No or not sure.
Are you member of any cleft palate association?	Yes No
What percentage of the time is an orthodontist present at your multidisciplinary clinic?	0-25%; 26-50%; 51-75%; 76-100%.
What percentage of your orthodontist's practice is devoted to the care of CLP patients	0-25%; 26-50%; 51-75%; 76-100%.
What is the most used tool for orthodontic diagnosis and treatment planning in your centre/office for CLP patients?	Panoramic and cephalometric radiograph; Cone beam computed tomography; Computed tomography; Other
How often do you perform pre-surgical orthopaedics in CLP patients in your clinical practice?	Always; Often; Sometimes; Never.
In which cleft phenotypes do you perform pre-surgical orthopaedics?	Cleft lip Cleft palate unilateral cleft lip and palate bilateral cleft lip and palate Pierre Robin sequence Other
What percentage of CLP patients have complications during orthodontic or surgical treatment?	0-25%; 26-50%; 51-75%; 76-100%.
What is the most frequent complication of CLP patients after surgical procedures:	Postoperative fistula; Postoperative infection; Postoperative airway complication; Revision; Other

Appendix 4.1. Survey questions and a list of possible answers

1 2 9 0 FACULDADE DE MEDICINA UNIVERSIDADE D COIMBRA

Question	Answer Options (If applicable)
In what country do you practise?	
How many years has your centre/office collaborated in CLP treatment?	
Please select where your centre/office is integrated:	Hospital environment; University environment; Private practice; University hospital environment;
What percentage of the time is an orthodontist present at your multidisciplinary clinic?	0-25%; 26-50%; 51-75%; 76-100%.
What percentage of your orthodontist's practice is devoted to the care of CLP patients?	0-25%; 26-50%; 51-75%; 76-100%.
Please tick the approximate percentage of your patient population in each category: 1. Dentofacial orthopaedics 2. Orthodontic treatment 3. Orthodontic-surgical treatment	0-25%; 26-50%; 51-75%; 76-100%.
How often do you perform pre-surgical orthopaedics on CLP patients in your clinical practice?	Always; Often; Sometimes; Never.
In which cleft phenotypes do you perform pre-surgical orthopaedics?	Cleft lip; Cleft palate; Unilateral cleft lip and palate; Bilateral cleft lip and palate; Pierre robin sequence; Other.
In which cleft phenotypes do you perform nasoalveolar moulding?	For patients with unilateral cleft lip and palate; For patients with bilateral cleft lip and palate; For all complete cleft lip and palate cases; Never.
What is the usual maxillary expansion protocol that your team uses in CLP patients?	Rapid maxillary expansion; Semi-rapid maxillary expansion; Slow maxillary expansion; Alt-RAMEC.
What appliance do you normally use for maxillary expansion?	Removable expansion plate; Quad helix; Hyrax/Hass; NiTi expander; Other tooth-borne appliance; Tooth-tissue-borne appliance; Bone-borne appliance.
In CLP cases with a missing maxillary lateral incisor, which treatment approach do you prefer?	Substitute the maxillary canines as the lateral incisor; Prosthetic replacement; No preference. Treatment approach depends on the clinical case; Other.
After the alveolar graft, how long do you wait until you start moving teeth to the newly grafted area?	1 month; 2 months; 3 months; 4 months; 5 months; 6 months; Other.
What alternative treatments do you use instead of secondary bone grafts?	Distraction osteogenesis; Tongue flap and secondary bone graft; BMP-2; None; Other.
Do you make other appliances (speech bulbs, etc.) for CLP patients?	Yes; No.
What percentage of CLP patients have complications during orthodontic or surgical treatment?	0-25%; 26-50%; 51-75%; 76-100%.
What is the most frequent complication of pre-surgical orthopaedics?	Interference with growth; Delaying surgery; Occlusion of the airway; Skin sores from the tape; Ulceration under a plate; Risk of infection under a plate; Feeding problems; Other.
What is the most frequent complication of orthodontics treatment?	Expansion relapse; Ulceration; Compromised oral hygiene; Phonetic problems; Mastication problems; Dysphagia; Other.
How far from your centre/office are the majority of your CLP patients?	0-50 km; 51-100 km; 101-150 km; >150 km.

How many appointments do CLP patients have at your centre/office each year?	0-3; 4-6; 7-12; >12.
How do CLP patients typically pay for orthodontic services?	National Health Service; Out-of-pocket expenses (self); Insurances; National Health Service and Self-pay; Insurances and Out-of-pocket expenses (self).
Are your patients satisfied with their current level of access to orthodontic services?	Dissatisfied; Neutral; Satisfied; Not sure.

Appendix 5.1. Ethics Committee (Reference: CE-071/2020)

1 2 9 0 FACULDADE DE MEDICINA UNIVERSIDADE D COIMBRA

COMISSÃO DE ÉTICA DA FMUC

Of. Refª **071-CE-2020**

Data **27/07/2020**

C/C aos Exmos. Senhores

Investigadores e co-investigadores

Exmo. Senhor

Prof. Doutor Carlos Robalo Cordeiro

Director da Faculdade de Medicina de

Universidade de Coimbra

Assunto: Pedido de parecer à Comissão de Ética - Projeto de Investigação autónomo (refª CE-071/2020).

Investigador(a) Principal: Inês Alexandre Neves Francisco

Co-Investigador(es): Francisco Fernandes do Vale e Maria Helena Fernandes

Título do Projeto: ***"Avaliação da qualidade de vida em doentes com fenda lábio-palatina sujeitos a tratamento ortodôntico"*.**

A Comissão de Ética da Faculdade de Medicina, após análise do projeto de investigação supra identificado, decidiu emitir o parecer que a seguir se transcreve:

"Parecer favorável; no entanto, sugere-se que a codificação dos participantes seja realizada com base em números de código e não nas suas iniciais; o tempo de resposta aos questionários deverá ser precisado (5 ou 10 minutos?) uma vez que se verifica inconsistência na informação fornecida".

Queira aceitar os meus melhores cumprimentos.

O Presidente,

Prof. Doutor João Manuel Pedroso de Lima

HC

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Appendix 5.2. OHIP-14 Questionnaire

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É convidado(a) a participar voluntariamente neste estudo, onde lhe será solicitado o preenchimento de um questionário. Os dados recolhidos são confidenciais e o seu uso é exclusivo para o presente estudo.

O preenchimento do questionário terá uma duração máxima de 5 minutos.

1. Data de preenchimento do questionário: ___/___/___
2. Iniciais do nome: _____
3. Sexo: Feminino__ Masculino__
4. Data de nascimento: ___/___/___
5. É portador de fenda labial e/ou palatina: Sim ___ Não ___
    a. Se sim, qual?
        i. Fenda bilateral lábio palatina ___
        ii. Fenda unilateral lábio palatina ___
        iii. Fenda Palatina ___
        iv. Fenda Labial ___
6. Dados sociodemográficos: _____

OHIP 14	Nunca	Quase Nunca	Ocasionalmente	Bastante Frequência	Muito Frequente
Dificuldade em pronunciar palavras/frases					
Paladar piorou					
Dores na boca					
Desconforto a comer					
Pouco à vontade					
Tensão					
Deixou de comer					
Interrompeu refeições					
Dificuldade em relaxar					
Vergonha					
Menos tolerante ou paciente					
Dificuldade em realizar atividades habituais					
Menos satisfeito com a vida em geral					
Incapacidade de funcionar					

Appendix 5.3. FIS Questionnaire

1290 FACULDADE DE MEDICINA UNIVERSIDADE DE COIMBRA

É convidado(a) a participar voluntariamente neste estudo, onde lhe será solicitado o preenchimento de um questionário. Os dados recolhidos são confidenciais e o seu uso é exclusivo para o presente estudo.

O preenchimento do questionário terá uma duração máxima de 5 minutos.

1. Data de preenchimento do questionário: ___/___/___
2. Iniciais do nome: _____
3. Sexo: Feminino__ Masculino__
4. Data de nascimento: ___/___/___
5. O seu filho(a) é portador de fenda labial e/ou palatina: Sim ___ Não ___
    a. Se sim, qual?
        i. Fenda bilateral lábio palatina ___
        ii. Fenda unilateral lábio palatina ___
        iii. Fenda Palatina ___
        iv. Fenda Labial ___
6. Dados sociodemográficos: _____

Durante os últimos 3 meses, devido aos dentes, lábios, boca ou maxilares do seu filho(a), com que frequência:	Nunca	1 a 2 Vezes	Algumas vezes	Frequentemente	Todos os dias
Atividade dos pais/familiares					
Você ou outro membro da família precisou de dispensa do trabalho (por ex. Dor,consultas, cirurgia)?					
Seu filho(a) requereu mais atenção da sua parte ou de outros membros da família?					
Você ou outro membro da família teve menos tempo para si ou para a sua família?					
Você ou outro membro da família teve o sono interrompido?					
Interferiu nas atividades da família em casa ou em outro lugar?					
Emoções dos pais					
Você ou outro membro da família sentiu-se perturbado?					
Você ou outro membro da família sentiu-se culpado?					
Você ou outro membro da família preocupou-se se o seu filho(a) iria ter menos oportunidades na vida (por ex. namorar, casar, ter filhos, ter emprego)					
Você ou outro membro da família sentiu-se desconfortável em lugares públicos (por ex. lojas e restaurantes) com o seu filho(a)?					
Conflito Familiar					
Seu filho(a) discutiu com você ou com outros membros da família?					
Seu filho(a) ficou com ciúmes de você ou de outros membros da família?					
Causou discordância ou conflito na sua família?					
Seu filho(a) culpou você ou outra pessoa da família?					
Dificuldades financeiras					
Causou dificuldades financeiras para a sua família?					

Appendix 6.1. AMSTAR2 checklist

1. Did the research questions and inclusion criteria for the review include the components of PICO?		
For Yes: ___ Population ___ Intervention ___ Comparator group ___ Outcome	Optional (recommended): ___ Timeframe for follow up	Yes ___ No ___
2. Did the report of the review contain an explicit statement that the review methods were established prior to the conduct of the review and did the report justify any significant deviations from the protocol?		
For Partial Yes: The authors state that they had a written protocol or guide that included ALL the following: ___ review question(s) ___ a search strategy ___ inclusion/exclusion criteria ___ a risk of bias assessment ___	For Yes: As for partial yes, plus the protocol should be registered and should also have specified: ___ a meta-analysis/synthesis plan, if appropriate, and ___ a plan for investigating causes of heterogeneity ___ a plan for investigating causes of heterogeneity	Yes ___ Partial Yes ___ No ___
3. Did the review authors explain their selection of the study designs for inclusion in the review?		
For Yes, the review should satisfy ONE of the following: ___ Explanation for including only RCTs ___ OR Explanation for including only NRSI ___ OR Explanation for including both RCTs and NRSI		Yes ___ No ___
4. Did the review authors use a comprehensive literature search strategy?		
For Partial Yes (all the following): ___ searched at least 2 databases (relevant to research question) ___ provided key word and/or search strategy ___ justified publication restrictions (e.g. language)	For Yes, should also have (all the following): ___ searched the reference lists / bibliographies of included studies ___ searched trial/study registries ___ included/consulted content experts in the field ___ where relevant, searched for grey literature ___ conducted search within 24 months of completion of the review	Yes ___ Partial Yes ___ No ___
5. Did the review authors perform study selection in duplicate?		
For Yes, either ONE of the following: ___ at least two reviewers independently agreed on selection of eligible studies and achieved consensus on which studies to include ___ OR two reviewers selected a sample of eligible studies and achieved good agreement (at least 80 percent), with the remainder selected by one reviewer		Yes ___ No ___
6. Did the review authors perform data extraction in duplicate?		
For Yes, either ONE of the following: ___ at least two reviewers achieved consensus on which data to extract from included studies ___ OR two reviewers extracted data from a sample of eligible studies and achieved good agreement (at least 80 percent), with the remainder extracted by one reviewer		Yes ___ No ___
7. Did the review authors provide a list of excluded studies and justify the exclusions?		
For Partial Yes: ___ provided a list of all potentially relevant studies that were read in full-text form but excluded from the review	For Yes, must also have: ___ justified the exclusion from the review of each potentially relevant study	Yes ___ Partial Yes ___ No ___
8. Did the review authors describe the included studies in adequate detail?		
For Partial Yes (ALL the following): ___ described populations ___ described interventions ___ described comparators ___ described outcomes ___ described research designs	For Yes, should also have ALL the following: ___ described population in detail ___ described intervention in detail (including doses where relevant) ___ described comparator in detail (including doses where relevant) ___ described study's setting ___ timeframe for follow-up	Yes ___ Partial Yes ___ No ___
9. Did the review authors use a satisfactory technique for assessing the risk of bias (RoB) in individual studies that were included in the review?		
RCTs For Partial Yes, must have assessed RoB from ___ unconcealed allocation, and ___ lack of blinding of patients and assessors when assessing outcomes (unnecessary for objective outcomes such as all-cause mortality)	For Yes, must also have assessed RoB from: ___ allocation sequence that was not truly random, and ___ selection of the reported result from among multiple measurements or analyses of a specified outcome	Yes ___ Partial Yes ___ No ___ Includes only NRSI ___
NRSI For Partial Yes, must have assessed RoB: ___ from confounding, and ___ from selection bias	For Yes, must also have assessed RoB: ___ methods used to ascertain exposures and outcomes, and ___ selection of the reported result from among multiple measurements or analyses of a specified outcome	Yes ___ Partial Yes ___ No ___ Includes only RCTs ___
10. Did the review authors report on the sources of funding for the studies included in the review?		
For Yes ___ Must have reported on the sources of funding for individual studies included in the review Note: Reporting that the reviewers looked for this information but it was not reported by study authors also qualifies		Yes ___ No ___
11. If meta-analysis was performed did the review authors use appropriate methods for statistical combination of results?		
RCTs For Yes, ___ The authors justified combining the data in a meta-analysis ___ AND they used an appropriate weighted technique to combine study results and adjusted for heterogeneity if present ___ AND investigated the causes of any heterogeneity		Yes ___ No ___ No meta-analysis conducted___
NRSI For Yes, ___ The authors justified combining the data in a meta-analysis ___ AND they used an appropriate weighted technique to combine study results, adjusting for heterogeneity if present ___ AND they statistically combined effect estimates from NRSI that were adjusted for confounding, rather than combining raw data, or justified combining raw data when adjusted effect estimates were not available ___ AND they reported separate summary estimates for RCTs and NRSI separately when both were included in the review		Yes ___ No ___ No meta-analysis conducted___
12. If meta-analysis was performed, did the review authors assess the potential impact of RoB in individual studies on the results of the meta-analysis or other evidence synthesis?		
For Yes, ___ included only low risk of bias RCTs ___ OR, if the pooled estimate was based on RCTs and/or NRSI at variable RoB, the authors performed analyses to investigate possible impact of RoB on summary estimates of effect		Yes ___ No ___ No meta-analysis conducted___
13. Did the review authors account for RoB in individual studies when interpreting/ discussing the results of the review?		
For Yes, ___ included only low risk of bias RCTs ___ OR, if RCTs with moderate or high RoB, or NRSI were included the review provided a discussion of the likely impact of RoB on the results		Yes ___ No ___
14. Did the review authors provide a satisfactory explanation for, and discussion of, any heterogeneity observed in the results of the review?		
For Yes, ___ There was no significant heterogeneity in the results ___ OR if heterogeneity was present the authors performed an investigation of sources of any heterogeneity in the results and discussed the impact of this on the results of the review		Yes ___ No ___
15. If they performed quantitative synthesis did the review authors carry out an adequate investigation of publication bias (small study bias) and discuss its likely impact on the results of the review?		
For Yes, ___ performed graphical or statistical tests for publication bias and discussed the likelihood and magnitude of impact of publication bias		Yes ___ No ___ No meta-analysis conducted___
16. Did the review authors report any potential sources of conflict of interest, including any funding they received for conducting the review?		
___ The authors reported no competing interests OR ___ The authors described their funding sources and how they managed potential conflicts of interest		Yes ___ No ___

Appendix 7.1. Search strategy for each of the databases

Database	Search Equation
Medline (via PubMed)	("Orthodontics"[Mesh] OR "Tooth Movement Techniques"[Mesh] OR "Orthodontic Brackets"[Mesh] OR " Tooth Movement" OR "Accelerating Orthodontic" OR "Surgery, Oral"[Mesh] OR "Alveolar Bone Grafting"[Mesh] OR "Post extraction Socket" OR "Socket Preservation" OR "Guided Tissue Regeneration"[Mesh] OR "Bone Regeneration"[Mesh] OR "Tissue Scaffolds"[Mesh] OR "Bone Transplantation"[Mesh] OR "Bone Remodeling"[Mesh] OR "Bone Substitutes"[Mesh]) AND ("Platelet-Rich Fibrin"[Mesh] OR "Platelet Rich Fibrin" OR "Fibrin rich in growth factors" OR "Platelet concentrate" OR "PRF" OR "Second generation platelet rich fibrina" OR "osteoinductive biomaterials")
Cochrane Library	(Mesh descriptor: [Orthodontics] OR Mesh descriptor: [Alveolar Bone Grafting] OR Mesh descriptor: [Bone Regeneration] OR tooth movement OR alveolar bone grafting) AND (Mesh descriptor: [Platelet-Rich Fibrin] OR PRF OR Platelet rich fibrin)
Web of Science Core Collection	TS=(platelet rich fibrin* OR platelet-rich fibrin* OR PRF* OR second generation platelet concentrate* OR platelet concentrate*) AND TS=(orthodontics* OR tooth movement* OR alveolar bone grafting* OR orthodontic brackets*)
EMBASE	("Orthodontics" OR "Orthodontic tooth movement" OR "Orthodontic device" OR "Alveolar Bone Grafting") AND ("Tissue regeneration" OR "bone regeneration") AND ("Platelet-rich fibrin" OR "platelet AND concentrate")

Appendix 7.2. Version 2 of the Cochrane tool for assessing risk of bias (RoB 2)

Bias domain and signalling question*	Response options		
	Lower risk of bias	Higher risk of bias	Other
Bias arising from the randomisation process			
1.1 Was the allocation sequence random?	Y/PY	N/PN	NI
1.2 Was the allocation sequence concealed until participants were enrolled and assigned to interventions?	Y/PY	N/PN	NI
1.3 Did baseline differences between intervention groups suggest a problem with the randomisation process?	N/PN	Y/PY	NI
Risk-of-bias judgment (low/high/some concerns)			
Optional: What is the predicted direction of bias arising from the randomisation process?			
Bias due to deviations from intended interventions			
2.1 Were participants aware of their assigned intervention during the trial?	N/PN	Y/PY	NI
2.2 Were carers and people delivering the interventions aware of participants' assigned intervention during the trial?	N/PN	Y/PY	NI
2.3 If Y/PY/NI to 2.1 or 2.2: Were there deviations from the intended intervention that arose because of the trial context?	N/PN	Y/PY	NA/NI
2.4 If Y/PY/NI to 2.3: Were these deviations likely to have affected the outcome?	N/PN	Y/PY	NA/NI
2.5 If Y/PY to 2.4: Were these deviations from intended intervention balanced between groups?	Y/PY	N/PN	NA/NI
2.6 Was an appropriate analysis used to estimate the effect of assignment to intervention?	Y/PY	N/PN	NI
2.7 If N/PN/NI to 2.6: Was there potential for a substantial impact (on the result) of the failure to analyse participants in the group to which they were randomised?	N/PN	Y/PY	NA/NI
Risk-of-bias judgment (low/high/some concerns)			
Optional: What is the predicted direction of bias due to deviations from intended interventions?			
Bias due to missing outcome data			
3.1 Were data for this outcome available for all, or nearly all, participants randomised?	Y/PY	N/PN	NI
3.2 If N/PN/NI to 3.1: Is there evidence that the result was not biased by missing outcome data?	Y/PY	N/PN	NA
3.3 If N/PN to 3.2: Could missingness in the outcome depend on its true value?	N/PN	Y/PY	NA/NI
3.4 If Y/PY/NI to 3.3: Is it likely that missingness in the outcome depended on its true value?	N/PN	Y/PY	NA/NI
Risk-of-bias judgment (low/high/some concerns)			
Optional: What is the predicted direction of bias due to missing outcome data?			
Bias in measurement of the outcome			
4.1 Was the method of measuring the outcome inappropriate?	N/PN	Y/PY	NI
4.2 Could measurement or ascertainment of the outcome have differed between intervention groups?	N/PN	Y/PY	NI
4.3 If N/PN/NI to 4.1 and 4.2: Were outcome assessors aware of the intervention received by study participants?	N/PN	Y/PY	NI
4.4 If Y/PY/NI to 4.3: Could assessment of the outcome have been influenced by knowledge of intervention received?	N/PN	Y/PY	NA/NI
4.5 If Y/PY/NI to 4.4: Is it likely that assessment of the outcome was influenced by knowledge of intervention received?	N/PN	Y/PY	NA/NI
Risk-of-bias judgment (low/high/some concerns)			
Optional: What is the predicted direction of bias in measurement of the outcome?			
Bias in selection of the reported result			
5.1 Were the data that produced this result analysed in accordance with a prespecified analysis plan that was finalised before unblinded outcome data were available for analysis?	Y/PY	N/PN	NI
Is the numerical result being assessed likely to have been selected, on the basis of the results, from:			
5.2 ... multiple eligible outcome measurements (eg, scales, definitions, time points) within the outcome domain?	N/PN	Y/PY	NI
5.3 ... multiple eligible analyses of the data?	N/PN	Y/PY	NI
Risk-of-bias judgment (low/high/some concerns)			
Optional: What is the predicted direction bias due to selection of the reported results?			
Overall bias			
Risk-of-bias judgment (low/high/some concerns)			
Optional: What is the overall predicted direction of bias for this outcome?			
Y=yes; PY=probably yes; PN=probably no; N=no; NA=not applicable; NI=no information			

Appendix 7.3. Robins-I tool for assessing risk of bias for non-randomised studies

Domain	Explanation
Pre-intervention	Risk of bias assessment is mainly distinct from assessments of randomised trials
Bias due to confounding	Baseline confounding occurs when one or more prognostic variables (factors that predict the outcome of interest) also predicts the intervention received at baseline. ROBINS-I can also address time-varying confounding, which occurs when individuals switch between the interventions being compared and when post-baseline prognostic factors affect the intervention received after baseline.
Bias in selection of participants into the study	When exclusion of some eligible participants, or the initial follow-up time of some participants, or some outcome events is related to both intervention and outcome, there will be an association between interventions and outcome even if the effects of the interventions are identical. This form of selection bias is distinct from confounding—A specific example is bias due to the inclusion of prevalent users, rather than new users, of an intervention.
At intervention	Risk of bias assessment is mainly distinct from assessments of randomised trials
Bias in classification of interventions	Bias introduced by either differential or non-differential misclassification of intervention status. Non-differential misclassification is unrelated to the outcome and will usually bias the estimated effect of intervention towards the null. Differential misclassification occurs when misclassification of intervention status is related to the outcome or the risk of the outcome, and is likely to lead to bias.
Post-intervention	Risk of bias assessment has substantial overlap with assessments of randomised trials
Bias due to deviations from intended interventions	Bias that arises when there are systematic differences between experimental intervention and comparator groups in the care provided, which represent a deviation from the intended intervention(s). Assessment of bias in this domain will depend on the type of effect of interest (either the effect of assignment to intervention or the effect of starting and adhering to intervention).
Bias due to missing data	Bias that arises when later follow-up is missing for individuals initially included and followed (such as differential loss to follow-up that is affected by prognostic factors); bias due to exclusion of individuals with missing information about intervention status or other variables such as confounders.
Bias in measurement of outcomes	Bias introduced by either differential or non-differential errors in measurement of outcome data. Such bias can arise when outcome assessors are aware of intervention status, if different methods are used to assess outcomes in different intervention groups, or if measurement errors are related to intervention status or effects.
Bias in selection of the reported result	Selective reporting of results in a way that depends on the findings and prevents the estimate from being included in a meta-analysis (or other synthesis).

Appendix 7.4. List of excluded studies

Study	Reason for exclusion
Alhasyimi et al.[24]	Animal study
Che et al.[25]	Review article
Dukka et al.[26]	Case Report
Janssen et al.[27]	Evaluates PRP and not PRF
Stasiak et al.[28]	Systematic review
Shetty et al.[29]	Trial registration
Subbalekha et al.[30]	Trial registration
Avinash et al.[31]	Trial registration
Tehranchi et al.[32]	Trial registration
Mazzone et al.[33]	Not orthodontic field
Shah et al.[34]	Technical note
Iskenderoglu et al.[35]	Case Report
Dimofte et al.[36]	Review article
Nadon et al.[37]	Evaluate a derivate of PRF
Aras et al.[38]	Case Report
Findik et al.[39]	Case Report

Appendix 8.1. Ethics Committee (Reference: CE-049/2019)

1 2 9 0 FACULDADE DE MEDICINA UNIVERSIDADE D COIMBRA

COMISSÃO DE ÉTICA DA FMUC

Of. Refª **048-CE-2019**
Data 27/2/**2019**

C/C aos Exmos. Senhores
Investigadores e co-investigadores

Exmo. Senhor
Prof. Doutor Duarte Nuno Vieira
Director da Faculdade de Medicina de
Universidade de Coimbra

Assunto: Pedido de parecer à Comissão de Ética - Projeto de Investigação autónomo (refª CE-049/2019).

Investigador(a) Principal: Inês Alexandre Neves Francisco

Co-Investigador(es): Francisco Fernandes do Vale, Maria Helena Fernandes, Isabel Amado e Margarida Mesquita

Título do Projeto: ***"Aplicação do L-PRF nos enxertos ósseos de doentes portadores com fenda lábio palatina".***

A Comissão de Ética da Faculdade de Medicina, após análise do projeto de investigação supra identificado, decidiu emitir o parecer que a seguir se transcreve:

"Parecer favorável".

Queira aceitar os meus melhores cumprimentos.

O Presidente,

Prof. Doutor João Manuel Pedroso de Lima

HC

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Overview of care in cleft lip and palate for orthodontic treatment

Inês Alexandre Neves Francisco

FACULDADE DE MEDICINA DENTÁRIA