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Direktor: Prof. Dr. med. dent.
Paul-Georg Jost-Brinkmann

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Cleft lip and palate: a variable clinical spectrum. Medical and dental considerations

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von
Theodosia N. Bartzela
Dr. med. dent., MSc., Ph.D.
geboren in Patras, Griechenland

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Dekan: Prof. Dr. Axel R. Pries
1. Gutacher: Herr Prof. Dr. Michael Wolf
2. Gutacher: Herr Prof. Dr. Christopher J. Lux

Dedicated to my parents

Contents

1. Introduction

1.1 Incidence of CLP according to ethnicity, cleft type, and sex

Orofacial clefts (OFC) are the most common developmental anomalies resulting from incomplete fusion of the facial processes. OFCs are classified according to the anatomical region involved and the degree of severity, which can range from minor involvement (microforms, i.e., a ridge running on the white of the lip, bifid uvula, or submucous CL) (Alvarez et al., 2014) to complete facial clefts, that is extending to the nose, cheeks, eyes, or even till the forehead (Phan et al., 2016).

There are three main phenotypes: cleft lip only (CLO), cleft palate only (CPO), and cleft lip and palate (CLP). CLP can be complete (CCLP) or incomplete (ICLP) and can be unilateral (UCLP) or bilateral (BCLP). The different phenotypes of CLP occur in different developmental stages (Shkoukani et al., 2013).

The primary palate (premaxilla) is formed during the 6th gestational week. The primary palate is a fusion of the medial nasal processes, the central part of the lip, and the four incisors' alveolar processes. The lip development occurs between the 6th and 8th gestational week, and the secondary palate (hard) between the 6th and 12th gestational week (Shkoukani et al., 2013).

1.1.1 Ethnicity

The incidence of orofacial clefts (OFCs) varies on ethnic and racial background, including the geographic area and the type of cleft (Mossey and Modell, 2012). The overall incidence of cleft lip and palate (CL/P) among Caucasians ranges from 0.91 to 2.69 per 1000 births (Vanderas, 1987).

In the EUROCAT special report, CLP patients' incidence was different among the European geographical regions. The lowest reported rate was in El Valles, Spain (0.63/1,000 births) and the highest in Finland (2.62/1,000 births) (EUROCAT, 2002) (Mossey and Modell, 2012).

In Germany, the prevalence was 14.52 in Saxony Anhalt and 23.85 in Mainz per 10,000 births (approximately 1/700 and 1/420 births, respectively) between 2001 and 2005 (Group, 2011). According to international data, the overall prevalence ranged from 3.4 to 22.9/10,000 births (Mossey and Castilla, 2003).

In another international database from 30 countries, 54 birth registries were included between 2000 and 2005 (Group, 2011). Data of 7.5 million births were presented. In total, 7,704

patients with CL/P have been registered. Among them, 7,141 were live births, 237 stillbirths, and 301 abortions, and in 25 of them, no information was available about the birth outcome. The overall prevalence of patients with CL/P was 9.92 per 10,000 births (approximately 1/1,000) (Group, 2011). Among these registries, South Africa had the lowest prevalence of CL/P (2.89/10,000), while Japan had the highest (20/10,000) (Group, 2011).

An international Database on Craniofacial Anomalies (ICDFA) was collected under the supervision of the WHO. Annual registration of 2 million births from 62 registries showed that the Indians, Aborigines, Scandinavians, and partially South Americans had the highest incidence rate. African, African American, and Southern Europe had the lowest incidence rate (Mossey, 2007).

In Singapore General Hospital, 1,105 new patients with CL/P were examined. The incidence of this cleft population (1985 to 1994) was 2.07 per 1,000 live births. Chinese was the group of patients with the highest incidence of 1.64 per 1000 compared to Indians and other ethnic groups (Yi et al., 1999).

In a meta-analysis based on data from low- and middle-income countries (LMICs) comprised 31,475,278 births, the birth prevalence of CL/P was 1.38 per 1000 (1/731) (Kadir et al., 2017). In this meta-analysis, the data and definitions provided in the literature were heterogeneous; therefore, a secondary analysis, based on a total of 75,627 births, had a prevalence of 0.75 per 1,000 births (Kadir et al., 2017).

OFC's overall incidence in New Zealand (in Maori and Pacific populations) between 2000 and 2010 was 1.79 per 1,000 live births. The Maori population had only patients with CPO, an increased rate of 2.37 per 1,000 live births (Thompson et al., 2016).

1.1.2 Cleft type

The most common type of cleft deformity is the CLP (Korolenkova et al., 2019). The clefts were less severe in patients with CLO and CPO (Jensen et al., 1988).

The prevalence per cleft type reported was 39% for patients with CLP, 33% with CPO, and 28% with cleft lip/alveolus CL/A (Luijsterburg and Vermeij-Keers, 2011). Similar is the reported distribution of cleft types in Denmark, where patients with CL/P were examined from 1976 to 1981. Among 602 patients, the reported cleft types were 39% for the patients with CLP, 34% for CLO, and 27% for CPO (Jensen et al., 1988).

Through the Human Genetic Program of the World Health Organization (WHO), data collected worldwide reported cleft-type distributions of 66.9% for CLP patients and 33.1% for

CLO. The prevalence reported by the WHO was 6.64 for patients with CLP and 3.28 for those with CLO per 10,000 births (Group, 2011).

The national survey in Japan showed similar results to the previously mentioned study. The incidence of 16,452 patients with a cleft was 6.2 for CLP, 4.2 with CL, and 2.8 with CPO per 10,000 births (Koga et al., 2016).

Contrarily, patients treated between 1998 and 2011 in Northern Finland had a higher incidence of CPO (68.7%) in comparison to the other cleft types (CLP (18.7%) and CL/A (12.6%)) (Lithovius et al., 2014). In agreement with (Lithovius et al., 2014), the data from the Czech Republic patients with CPO had the highest incidence (0.68/1,000), followed by the patients with CLP (0.57/1,000) and CLO (0.39/1,000) evaluated among 1,471,789 births between 1994 and 2008. The overall incidence was 1.64 per 1,000 live births (1 in 600) (Urbanova et al., 2013).

Regarding the distribution of the different cleft types, there is a significant variation in patients afflicted with CL/P compared to those with CPO in the various ethnic groups. Africans and African descent are more commonly affected by CLO, while Northern Europeans (mainly Finland and Scotland), Asians, Native Americans, and Aboriginal Australians are affected by CL/P (Mossey and Modell, 2012). Furthermore, bilateral cleft lip and palate (BCLP), the most severe type of cleft, was observed in only 7% of the 3,616 cleft patients (Sivertsen et al., 2008a).

Concerning the cleft's sidedness, the left-sided patients were almost 2 out of 3 (Korolenkova et al., 2019). In Northern Finland, where the incidence of patients with CL/P is higher than in other European countries, left-sided clefts were observed even more frequently (82% of patients) than right-sided clefts (18%) (Lithovius et al., 2014). The CL/P's left predominance has been observed in the offspring of affected and non-affected-family members (Sivertsen et al., 2008b). The cleft's severity was not related to the sidedness, and patients with BCLP had almost the same severity on both sides (Sivertsen et al., 2008a).

1.1.3 Sex distribution and clefts

Sex distribution in Danish infants born with clefts was 61% males and 39% females (Jensen et al., 1988). Males were affected more frequently with CLO and CL/P (Urbanova et al., 2013), with a stable male-to-female ratio of 1.75:1. Contrarily, females have a slight predominance on CPO compared to males. The male-to-female ratio was 0.97:1 in 1998,

which decreased to 0.59:1 in 2007 (Matthews et al., 2015). Girls were more likely to have severe clefts and other congenital malformations and syndromes (Sivertsen et al., 2008a).

1.2 Etiology and risk factors

The etiology of nsCL/P is multifactorial. Both genetic and environmental factors are involved, but the pathogenetic mechanism is not elucidated (Raut et al., 2019). More than 300 genes have been identified for the syndromic and non-syndromic phenotypes (Bartzela, 2011; Maili et al., 2020).

Nevertheless, the maternal genes and environmental triggers are responsible for the pathogenesis and the phenotypic variability of patients with CL/P (Jugessur et al., 2010). The phenotypic differentiation in twins indicated the contribution of environmental factors (Grosen et al., 2011). Environmental contributors such as maternal risk factors like alcohol, smoking, diabetes, maternal age at conception, folate and zinc deficiency, and teratogens such as valproic acid, phenytoin, retinoic acid, chemicals, pesticides, and occupations related to leather and health care are contributing to the pathogenesis of the clefts (Shkoukani et al., 2013). Vitamins like A, B2, B6, B12, folic acid, and zinc (Marini et al., 2016) showed modest risk protection when used for maternal supplement nutrition around conception time (Wilcox et al., 2007).

Furthermore, the pathogenic mechanisms for nsCL/P and CPO (Ludwig et al., 2017) are distinct, and a strong genetic component of CPO has been emphasized (Dixon et al., 2011). The environmental contributors have been studied in twin patients (Grosen et al., 2011). A Danish study examined 39 non-syndromic CL/P twin patients. According to the findings of the study, CL/P's concordance rate was 60% in monozygotic and 10% in dizygotic twins (Christensen and Fogh-Andersen, 1993). These findings accentuate the environmental component of the pathogenic mechanism.

Interestingly, genetic variants involved in the "normal" facial phenotype of the non-affected individuals are also responsible for the nsCL/P phenotype (Howe et al., 2018). Minor variables on risk alleles involved in clefting, such as the *VAX1*, may affect smell and vision (Boehringer et al., 2011). Mutations in *PBX1, PBX2, TP63, BMP4, PBX-WNT-TP63-IRF6* regulatory pathways and interactions between the *PBX1* and *WNT9B* contribute to the nsCLP etiology (Maili et al., 2020). Furthermore, mutations of the interferon regulatory factor 6 (*IRF6*) gene have been associated not only with nsCL/P (Leslie et al., 2016) but also with the sCLPs, such

as the van der Woude syndrome (Khandelwal et al., 2017) and other associated dental, epidermal and genital malformations (Phan et al., 2016). Mutations in other genes, such as *TBX22, P63, BMP4, FGFR1,* and *FGFR2,* are involved in the pathogenesis of syndromic clefts (Shkoukani et al., 2013)**.** Epigenetic factors (Takahashi et al., 2018) or genes that contribute to the laterality of organ development, such as e.g., the BCOR-gene in the Occulo-facio-cardio-dental syndrome (OFCD), may contribute to the laterality of the cleft (Hilton et al., 2007; Hilton et al., 2009). The severity of the cleft plays a pivotal role in the recurrence rate in the offspring (Mangold et al., 2011).

CLP's risk among siblings is 30 times higher than the general population's prevalence (Christensen and Fogh-Andersen, 1993). A gene-specific mutation or the associated *IRF6* haplotype could raise the recurrent familial risk to much higher values (Zucchero et al., 2004).

1.3 Associated malformations and syndromes

Patients with severe CL/P were more likely to have malformations in other systems (Group, 2011). The National Birth Defects Prevention Network (NBDPN) in the United States and International Clearinghouse for Birth Defects Surveillance and Research (ICBDSR) worldwide reported a prevalence of CLP of 6.64 and CL 3.28 per 10,000. In their data, 5918 patients (76.8%) had no associated malformation, 1,224 (15.9%) had associated abnormalities, and 562 (7.3%) had an associated recognized syndrome (Group, 2011).

1.3.1 Associated malformations

The prevalence of associated congenital malformations in orofacial clefts (OCs) is discussed in many studies, and the results vary widely. The reported prevalence of these anomalies ranges between 3% and 63.4% (Fraser, 1970). The Dutch association for clefts and craniofacial anomalies (NVSCA) collected data from 1997 to 2006, and 23% of 3,512 patients had family members with associated malformations (Luijsterburg and Vermeij-Keers, 2011).

Furthermore, the severity of CL/P is associated with the degree of severity of the congenital malformations of other organs or systems (Group, 2011). Therefore, patients with CLP had more frequent associated malformations (34%) than the ones with CLO (20.8%) (Calzolari et al., 2007). An increased incidence of associated malformations has been reported in patients with BCLP, as it has been considered the most severe CLP phenotype (Hagberg et al., 1998). Following the previous reports, associated malformations have been reported in 35% of patients

with BCLP and 24% with UCLP (Milerad et al., 1997). In a prenatal diagnostic screening, more fetuses with BCLP (72%) were diagnosed with a congenital malformation than those with UCLP (48%) (Bergé et al., 2001).

The fact that CPO and CLP are distinct regarding their embryologic origin differentiates their occurrence with associated malformations (Wen, 2015). Therefore, the existence of any type of associated malformations was 19.8% for CL/P vs. 41.3% for CPO patients (Koga et al., 2016).

Data from southwestern Sweden collected for 30 years from children with different CPO phenotypes revealed associated congenital malformations in 34% of these patients (Chetpakdeechit et al., 2010). Patients with complete CPO had 1.7 times increased risk of co-occurrence with an associated anomaly than the less severe CPO phenotypes, such as the incomplete or the submucous CPO (Chetpakdeechit et al., 2010). The most common associated malformations were heart defects and intellectual impairment (Chetpakdeechit et al., 2010).

Additionally, various congenital anomalies have been observed in the head (68% in the mandible) and neck area (Luijsterburg and Vermeij-Keers, 2011), cardiovascular (24-51%) and urogenital systems, vertebral column, and extremities, as well as in ears and eyes (anophthalmia/microphthalmia, etc.) (Stoll, 2000). Additional clinical features were ectrodactyly, hypogonadism, ectodermal and musculoskeletal anomalies, hearing, and mental impairment (Bartzela et al., 2017).

In an Australian population, 31% of children with CL/P and 61% with CPO had associated congenital anomalies, which is higher than the previously presented data (Dentici et al., 2015).

In the Cleft Palate-Craniofacial Unit of the Radboud University Nijmegen Medical Centre, from 149 patients with a complete BCLP (cBCLP), we included available data of 133 patients. Thirty-one patients (23%) had a single additional malformation, and 33 patients (25%) had several other congenital anomalies (Kouwenberg M, 2010). According to the data of the NVSCA (Dutch Association for cleft palate and craniofacial anomalies), of the 3512 patients included, 23% of them had associated congenital anomalies. Among them, 10% had associated malformations. Forty different malformations were identified only in the craniofacial and neck area, 68% in the mandible. 13% of the malformations were observed in various organs (Luijsterburg and Vermeij-Keers, 2011). Family members of patients with CPO had less common associated congenital anomalies than the other cleft phenotypes (Luijsterburg and Vermeij-Keers, 2011).

1.3.2 Associated syndromes

Patients with an OFC can have an associated syndrome or a monogenic, chromosomal, or related extrinsic factors disorder (Kouwenberg M, 2010). Hence, more than 600 syndromes have been identified with CL/P (Dixon et al., 2011), of which more than 400 have been associated with CPO. 30% of patients with CL/P (Stanier and Moore, 2004) and 50% of patients with CPO are afflicted with a syndrome (Murray, 2002). Patients with CPO were diagnosed most frequently with the Pierre Robin sequence, and CL/P patients mostly with trisomy-21 or trisomy-13. In more severe cases, patients with Pierre Robin sequence (PRS) can be diagnosed with multiple congenital anomalies (PRS-plus) besides the cardinal characteristics of the PRS or other syndromes (sPRS). The etiopathogenesis of non-syndromic CLP (nsCLP) from sCLP patients has been differentiated. According to the data of the NVSCA (Dutch Association for CL/P and craniofacial anomalies), in 24% of the children with a positive family history of OFC, an associated syndrome was diagnosed (Luijsterburg and Vermeij-Keers, 2011).

The van der Woude syndrome (VWS) is the most common syndrome associated with CL/P and accounts for 2% of these patients (Bartzela et al., 2017). The second most common syndrome associated with CL/P is the 22q11.2 deletion syndrome, also known as Velocardiofacial, Shprintzen, or DiGeorge syndrome, with a prevalence of 25 – 50 /100,000 (Bartzela et al., 2017). Other common syndromes in association with CL/P are the EEC-syndrome (Ectrodactyly, ectodermal dysplasia, and cleft lip/palate syndrome), the Pierre Robin Sequence, the Kallmann and Kabuki syndrome (Bartzela et al., 2017), Apert, Oculo-Auriculo-Vertebral spectrum (Wieckowska et al., 2015). In many of these syndromes, vital functions are severely affected and considered life-threatening conditions. Patients with sPRS have an almost 10% mortality rate related to respiratory failure or neurological disorders (Logjes et al., 2018).

1.4 Tooth agenesis and CL/P

Dental anomalies are a common finding in patients with CL/P; among them, tooth agenesis (TA) and supernumerary teeth have the highest prevalence (Lehtonen et al., 2015). The cleft's severity is associated with more severe tooth agenesis patterns (Bartzela et al., 2010b; Bartzela et al., 2013). Nevertheless, TA occurs not only on the affected side but also outside the cleft in the maxilla and mandible (Bartzela et al., 2013). Hypodontia is similarly prevalent in subjects with CPO independent of the family history associated with CL/P but varies widely.

Furthermore, hypodontia's prevalence is very high in syndromes associated with CL/P, such as the van der Woude syndrome and the Pierre Robin sequence. Factors possibly accounting for TA are related to embryogenic disruption or iatrogenic interventions in the cleft area (Lekkas et al., 2000). The upper lateral incisor is the most susceptible tooth to iatrogenic injury. Iatrogenic factors that might disrupt tooth formation are neonatal hard palate closure or primary bone grafting (Bartzela et al., 2013). More teeth are congenitally missing in the maxilla than the mandible. TA outside the cleft area is related to genes, and genomic loci in a possible cellular and molecular synergy (Phan et al., 2016) related to palatogenesis (van den Boogaard et al., 2000).

A recent literature review of combined phenotypes of CL/P and TA reported nine genomic loci and 26 candidate genes (Phan et al., 2016). The candidate genes reported in the literature present a different degree of evidence, and the phenotypic severity in both the CL/P and TA varies widely (Phan et al., 2016). The cleft side is more frequently affected than the non-cleft side (Bartzela et al., 2013). Co-occurrence of TA and CL/P in humans has been associated with mutations of *IRF6* or *TP63*, both in syndromic and non-syndromic forms (Kondo et al., 2002). The van der Woude syndrome (VWS), in 68% of the patients, is attributed to a variant in the *IRF6* gene (Yu et al., 2020). *MSX1* stop mutation has been identified in afflicted family members with CL/P and TA (van den Boogaard et al., 2000). Mainly, *MSX1* mutations are associated with the non-syndromic co-existence of OFCs and TA and account for the Wolf-Hirschhorn and Witkop syndromes (Liang et al., 2016). The PAX9 protein interaction with MSX1 has been associated with human OFC and TA, inside and outside the cleft region. Mutation in TGFβ3 is associated with TA outside the cleft (Phan et al., 2016).

1.5 Orthodontic considerations in patients with CL/P

The orthodontist has a challenging role in guiding dental and craniofacial development. The majority of the patients with CL/P (92.3% males and 71.5% females) have a severe form of malocclusion manifested already in the primary dentition (Bartzela et al., 2013),(Hujoel and DeRouen, 1995). Posterior crossbite and midline deviation are the most frequent occlusal findings (Hujoel and DeRouen, 1995). Additionally, several dental anomalies, including variations in dental number, shape, size, path, and eruption timing, complicate the orthodontic treatment (Bartzela et al., 2013; Rizell et al., 2020). Hypoplastic or atypical-shaped laterals are observed on the affected and non-affected sides (Bartzela et al., 2013). A delay of tooth

formation of the permanent teeth varies from 0.3 to 0.7 years in the maxilla and mandible (Ranta, 1986).

Infant orthopedics (IO) for perinatal care and, later, treatment for dental malocclusion and maxillofacial development should be encountered. IO's favorable effect on craniofacial growth has been debated over the last few years (Bongaarts et al., 2009). Crossbite correction is performed during the early mixed dentition period and before bone grafting. After the eruption of at least the first premolar, the orthodontic treatment with a multibracket appliance (MBA) can start (Bartzela, 2019). Rotated permanent maxillary incisors can be aligned earlier to eliminate premature contacts or if the aesthetics are an early consideration (Bartzela et al., 2019).

1.5.1 External apical root resorption during orthodontic treatment in patients with CL/P

Patients with CL/P are usually characterized by a narrow and asymmetric maxilla, midline deviation, and soft and hard tissue deficiency along the cleft area. Special periodontal conditions, hypoplastic, missing, and malformed teeth, especially into the cleft, are common findings (Dewinter et al., 2003). Furthermore, patients with CL/P have additional predisposing factors indicating a higher incidence of external apical root resorption (EARR).

Patient's intrinsic factors predisposing to EARR can be trauma (Malmgren et al., 1982) or preexisting root resorption (Massler and Malone, 1954; Brezniak and Wasserstein, 1993), or abnormal root ending (Kjær, 1995; Sameshima and Sinclair, 2001; Al-Qawasmi et al., 2003; Nanekrungsan et al., 2012a). Incompletely developed teeth (Rosenberg, 1972) or endodontically treated (Spurrier et al., 1990) have a protective effect over EARR. Orthodontic techniques (standard edgewise vs. straight-wire (Mavragani et al., 2000) or aligner vs. MBA (Iglesias-Linares et al., 2017) or even different types of brackets seem that do not affect the EARR (Pandis et al., 2008a; Leite et al., 2012; Jacobs et al., 2014; Aras et al., 2018a). Nevertheless, the use of heavy orthodontic wires (Alzahawi et al., 2014), the force magnitude (Weltman et al., 2010), treatment duration (Fox, 2005; Årtun et al., 2005), the intermaxillary elastics (Simplicio et al., 2012), and the amount of orthodontic movement (OTM) (Fox, 2005) play a role in an EARR which is of clinical significance (Bartzela et al., 2019).

1.5.2 Diagnostic tools for patients with CL/P

Various diagnostic tools have been developed to evaluate the treatment outcome of dental casts of patients with CL/P. These yardsticks have been used to rate dental arch relationships (Huddart and Bodenham, 1972) and predict the maxilla's skeletal development compared to the mandible (Ozawa et al., 2011). Moreover, these yardsticks have been widely used for the treatment outcome evaluation in intercenter studies (Bartzela et al., 2010a; Bartzela et al., 2011) and as an indicator of the treatment effectiveness in intracenter assessments (Mars et al., 2006; Mars et al., 1987).

Nevertheless, traveling with plaster casts for evaluation intercenter treatment outcome studies is not always feasible. Using digital models or photographs of dental casts instead of plaster casts for the treatment outcome assessment in children with complete bilateral cleft lip and palate (cBCLP) has been proved reliable (Leenarts et al., 2012). Isolated dental arches and dental occlusion are rated in sagittal, transverse, and vertical dimensions. The anteroposterior relationship is significant because it is a predictor of the treatment need. Dental compensations are also taken into consideration (Ozawa et al., 2011).

The yardstick for assessing the plaster models in patients with BCLP has a five-grade scale. The evaluations are made in the primary, mixed, and early permanent dentition (6-, 9-, 12-years of age, respectively).

Furthermore, yardsticks have been developed to evaluate the nasolabial appearance (Asher-McDade et al., 1992). Photographs of patients with CL/P are evaluated in different views (frontal, profile, and worm’s eye) separately. The following landmarks are assessed for the red of the lip, the length of the upper lip, and the columella, the shape of the nose’s tip. A final rating of the nasolabial appearance for children with CL/P occurred at 6-, 9-, and 12-years, as previously mentioned for the dental casts.

1.6 Objectives of the thesis

This retrospective intercenter study was based on patient data collected from three European cleft centers (Radboudumc, Nijmegen, the Netherlands; Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Campus Mitte, Germany).

Patients with CL/P present a broad spectrum of phenotypic variability and need long, interdisciplinary treatment. Multidisciplinary interaction, early diagnosis, and therapeutic

guidance of patients with CL/P, with or without associated malformations or syndromes, is challenging.

This thesis's primary goal is to present the clinical phenotypes, focusing mainly on these patients' craniofacial and dental structures. Furthermore, to provide an overview of the interdisciplinary team's comprehensive treatment approach, focusing on the craniofacial and dental structures. Moreover, to enhance the involved team members' communication to implement individualized, integrated, and long-term treatment pathways (Bartzela, 2019).

Therefore, the aim of this thesis was to identify factors that may influence the final treatment outcome through this cross-sectional and longitudinal evaluation of dental arches and craniofacial morphology.

The specific aims of the study were to present:

- The incidence of patients with CL/P and their distribution according to the cleft type, laterality, and sex
- The prevalence of associated anomalies or syndromes in patients with CL/P in two different University clinics
- The family history and the pedigrees of these patients and associated with the condition
- Tooth agenesis patterns and their prevalence in patients with complete unilateral cleft lip and palate (cUCLP)
- The reliability of using digital models or photographs of dental casts as an alternative to plaster casts for rating dental arch relationships in children with complete bilateral cleft lip and palate (cBCLP).
- The incidence of external apical root resorption (EARR) in the anterior maxillary teeth of patients with cUCLP during orthodontic treatment and to evaluate the variable that may influence the development of EARR.

Due to the low incidence of cBCLP compared to the rest of the cleft population, published literature on cBCLP is scarce (Kouwenberg et al., 2011). Hence, our knowledge of treatment outcomes in patients with cBCLP is somewhat limited. Relatively few longitudinal studies on BCLP in general and on dental arch dimensions and relationships, and craniofacial morphology have been published.

Comparing treatment outcomes between cleft centers could elucidate the importance of factors like ethnic characteristics and specific treatment interventions and give the team an insight into the effectiveness of their treatment. Following patient samples longitudinally may

provide important information regarding the impact of different treatment approaches on craniofacial growth and dental arch development. Retrospective studies (Bartzela et al., 2021; Kouwenberg et al., 2011) are subjected to selection, exposure, and outcome assessment biases. The studies presented, though, are based on a long-term, meticulously collected database from interdisciplinary teams.

2.1 [Associated congenital malformations in patients with complete bilateral cleft lip and palate]

Kouwenberg M., Draaisma J.M., Kuijpers-Jagtman A.M., Bartzela T.

Tijdschrift voor kindergeneeskunde 2011;78:13-17
DOI: 10.1007/s12456-010-0004-5

Summary
Orofacial clefts can be associated with a syndrome (of known or suspected pathogenesis or etiology) or a chromosomal, monogenic, or disorder triggered by exogenous factors. Associated congenital anomalies and syndromes have been reported more often in children with bilateral cleft lip and palate (BCLP) than in other types of orofacial clefts. The reported incidence varies from 7.5 to 63.4% (6, 7). Nevertheless, data on syndromes in this group of patients are rare. We present a retrospective study on the incidence of other congenital anomalies and syndromes in patients with complete BCLP (cBCLP).

This retrospective study aimed to describe the incidences of congenital anomalies and associated syndromes in children with BCLP treated by Radboud University Nijmegen Medical Centre's cleft team (Nijmegen, the Netherlands).

Patients and Methods: All patients with cBCLP treated by the cleft team of Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands, born before the 1st of January of 2003, were included in this retrospective study. A clinical geneticist examined all these children when they were six months of age. If necessary, genetic (chromosomal or DNA examination) and/or metabolic analysis were performed, depending on the patient. All patients were examined again for associated malformations or syndromes when they were three years old and were followed up by the cleft team. All patients with cBCLP and congenital anomalies, syndromes, or chromosomal deviations were included in the study. These patients' reported congenital anomalies were classified according to their origin as the cardiovascular, respiratory, gastrointestinal, urogenital, central nervous system, vertebral column, extremities, facial, ear, and eye. Furthermore, syndromes and other congenital anomalies were registered.

Results: Finally, 133 of these children with cBCLP were included in our study. Sixty-four (48%) out of the 133 patients with cBCLP had a congenital malformation reported before the children were three years old. Sixty-nine of these patients (52%) had no congenital malformation. Seventeen (26%) of these patients were females. The most common congenital malformations reported were in the heart (8.3% out of 133), urogenital system (10.5%),

extremities (9.0%), and other anomalies (12.8%). In 19 patients, associate malformations have been diagnosed when the children were older than three years of age. These anomalies were mainly dental (deviations in size, shape, or agenesis). In only one child older than three years of age, congenital anomalies were diagnosed besides dental developmental abnormalities. This patient was diagnosed with ectropion of the eyelid, ear tube atresia, and developmental retardation. In 12 out of the 133 patients (9%), a syndrome was diagnosed.

Moreover, a syndrome was suspected in three other children, but no further information or follow-up data were provided. Seven (58%) of the 12 children with a syndrome were females. The female predominance of patients with BCLP and an associated syndrome, in comparison to males, was significant ($p = 0.005$). Fifteen of the children with multiple congenital anomalies had most probably a suspected syndrome that was not identified. Twelve out of 33 (36.4%) of these children with various congenital anomalies were diagnosed with a syndrome. Nine of the children with a syndrome had multiple congenital anomalies.

Conclusions: Children with a cBCLP often have other associated congenital malformations. Specific syndromes have also been diagnosed in 9% (out of 133) of this group of children. Therefore, pediatricians should give particular attention to this group of children for associated congenital malformations or syndromes.

Note: This publication has been translated into English by Dr. Dr. T. Bartzela for this habilitation thesis (pages: 23-30).

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ARTIKELEN

2.1 [Associated congenital malformations in patients with complete bilateral cleft lip and palate]

Kouwenberg M., Draaisma J.M., Kuijpers-Jagtman A.M., Bartzela T.

Tijdschrift voor kindergeneeskunde 2011;78:13-17

Translated in English for this thesis by Dr. Dr. Bartzela

Abstract

Introduction: In patients with complete bilateral cleft lip and palate (BCLP), other congenital anomalies are frequently reported. Data on syndromes in this group of patients are rare. We present a retrospective study on the incidence of other congenital anomalies and syndromes in patients with complete BCLP.

Design, setting, and patients: Data on other congenital anomalies and syndromes (reported from birth till three years of age) were collected from records of patients born before 2003, treated in the Cleft Lip and Palate Centre of the Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands.

Results: Of the records of 149 patients with complete BCLP, the data of 133 of them were included in this study. Thirty-one patients (23%) had a single additional anomaly, and 33 (25%) had several other abnormalities. The most frequently reported anomalies were of cardiac (8.3%), urogenital origin (10.5%), abnormalities of the extremities (9.0%), and the face (14.3%). In 12 patients (9.0%), a syndrome (i.e., chromosomal etiology) was diagnosed. In three other patients, a syndrome was suspected. Seven of the 12 patients diagnosed with a syndrome were females ($p = 0.05$).

Discussion: Our results are consistent with previously reported data on associated congenital anomalies in patients with BCLP. Very little data is present on syndromes in patients with complete BCLP. We cannot adequately compare our data with those of previous studies. Female predominance in syndromes has not been reported before.

Conclusion: In patients with complete BCLP, other congenital anomalies are frequently observed. In these patients, pediatricians should also be alerted for the co-occurrence of a syndrome.

Introduction

Unilateral (UCLP) or bilateral (BCLP) cleft lip and palate is the most common congenital orofacial malformation. In the Netherlands, 2.03 out of 1,000 children are born with an orofacial cleft (1). The reported incidence varied in different European countries from 0.91 to 2.69 per 1000 births (calculated in live or stillborn infants). (2) The incidence of children with BCLP is about 0.2 per 1,000 live births. (3) It is twice as frequent in males than in females (4, 5).

Associated congenital anomalies have been reported more often in children with BCLP than in other types of orofacial clefts or the general population. The reported incidence varies from 7.5 to 63.4% (6, 7). The most common congenital anomalies in children with BCLP are cardiovascular, gastrointestinal, urogenital, in the skeleton (most common in vertebral column), extremities (7), ears, and eyes (7, 10, 11).

Patients with cleft lip and palate suffer more congenital anomalies (25-28%) than patients with isolated cleft palate (22%) or with cleft lip (8-10.2%) (3, 8).
Milerad *et al.* reported in their study associated congenital malformations in 35% of patients with BCLP and 24% in patients with UCLP (3). In a prenatal sonographic diagnosis in fetuses with an orofacial cleft, the associated anomalies were more frequent in fetuses with bilateral clefts (72%) than in those with unilateral clefts (48%) (9).

Orofacial clefts can be associated with a syndrome (of known or suspected pathogenesis or etiology) or a chromosomal, monogenic, or exogenous factors disorder. Reported associated syndromes in patients with a cleft are about 5.4-12.6%. The most common associated syndromes in infants with an orofacial cleft are trisomy 13, 18, and 21 and the van der Woude syndrome (3, 8). Publications on patients with BCLP and associated malformations are rare.
Lilius reported an incidence of 4.4% associated syndromes of chromosomal etiology in 113 children with BCLP (10).

This retrospective study aimed to describe the incidences of congenital anomalies and associated syndromes in children with BCLP, treated by Radboud University Nijmegen Medical Centre's cleft team (Nijmegen, the Netherlands). These findings have been compared with relevant published data.

Patients and Methods

All patients with complete BCLP (cBCLP) treated by the cleft team of Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands, born before the 1st of January 2003, were included in this retrospective study. A clinical evaluation was made when the patients were three years old, and the interdisciplinary team followed them up. A geneticist examined all these children when they were six months of age, and if necessary, the children were followed up. Genetic analysis was performed (chromosomal or DNA examination). Evaluation of the patient's metabolism and/or radiographic examination was considered depending on the patient. All patients with cBCLP and congenital anomalies, syndromes, or chromosomal deviations were included in the study. These patients' congenital anomalies were classified according to their origin to the cardiovascular, respiratory, gastrointestinal, urogenital, central nervous system, vertebral column, extremities, facial, ear, and eye. Furthermore, developmental deviations, syndromes, and other congenital anomalies were also included.

SPSS 12.0.1. was used for the statistical analysis. Fisher Exact test was used for male and female predominance with cBCLP and associated anomalies.
The statistical significance was determined at $p \leq 0.05$.

Results

Children with cBCLP were included in this study. The initial group of patients consisted of 149 children. Data were missing in 16 of these children; therefore, they were excluded from the study. Finally, 133 of these children with cBCLP were included in our study. Forty-one (31%) were females, and 92 (69%) were males. Sixty-four (48%) out of the 133 patients with cBCLP had a congenital malformation reported before the children were three years old.

Sixty-nine of these patients (52%) had no congenital malformation. Seventeen (26%) of these patients were females. The most common congenital malformations reported were in the heart (8.3% out of 133), urogenital system (10.5%), extremities (9.0%), and other organs (12.8%). All reported congenital deviations are presented in Table 1. In 19 patients have been reported, anomalies were diagnosed when the children were older than three years of age. These anomalies were mainly dental (tooth agenesis or tooth shape deviations). In only one child older than three years, congenital anomalies were diagnosed besides dental abnormalities. This patient was diagnosed with ectropion of the eyelid, ear tube atresia, and developmental retardation. In 12 out of the 133 patients (9%), a syndrome was diagnosed.

Moreover, a syndrome was suspected in three other children, but no further information or follow-up data was provided. Seven (58%) of the 12 children with a syndrome were females. Compared to males in patients with BCLP and an associated syndrome, the female predominance was significant (p = 0.005). Fifteen of the children with multiple congenital anomalies had most probably a suspected syndrome that was not identified. Twelve out of 33 (36.4%) of these children with multiple congenital anomalies were diagnosed with a syndrome. Nine of the children with a syndrome had multiple congenital anomalies. The diagnosed syndromes of these children with cBCLP are presented in Table 2.

Discussion

In this retrospective study, we evaluated the congenital deviations of children with cBCLP. We reported the congenital malformations of these children followed for the first three years of their life. We decided to evaluate the diagnosed congenital malformations of cBCLP patients, the first three years of their lives. According to previously published data, most congenital malformations were diagnosed until the first three years of patients' lives (12). Even though the patients were followed up until the fourth year, only 2-3% additional children were diagnosed with a congenital malformation (12). After 12 years of follow-up, only an extra 5% of these patients were diagnosed with a congenital malformation, which was not diagnosed in their first three years of life (12).

In total, 64 (48%) of these patients were diagnosed with congenital malformation. The most common congenital malformations diagnosed were mainly on the face, extremities, urogenital and cardiovascular system. Our results agree with previously published data by Lilius (10) (presented in Table 3). Compared with our study group of patients, 35% of the children had congenital malformations (3). The follow-up period, though, was longer than in our group (3). Children with BCLP had congenital malformations in the head, neck, face, and extremities (7, 10). Vertebral column malformations have been reported in 16.3% of another group of patients with BCLP (11). Congenital heart malformations have been registered in 12.5% of children with BCLP (13). Our study is the first to report developmental deviations (dysmaturity and failure to thrive, in total 12.8%) and cervical vertebral malformations in children with BCLP.

An orofacial cleft is more often in males than females (male/female ratio 1.2). In our study, male patients with cBCLP are predominant than females (male/female ratio 2.2). Our results follow previously published data in patients with BCLP, where the male/female ratio

was about 2.1-2.2 (4, 5). Roughly a male/female ratio in patients with BCLP with a syndrome or chromosomal disorder has not been reported in the scientific literature. In this group of patients, we found associated congenital anomalies or syndromes in the male/female ratio of 0.71 (p-value=0.05). More studies are required to evaluate the prevalence of (specific) syndromes or chromosomal anomalies and the sex predisposition in patients with BCLP. The increased incidence of multiple congenital anomalies in patients with BCLP points to a critical medical examination in the first three years of patients' lives should be considered.

From an embryological perspective, the neural plate and neural crest cells play a pivotal role in forming the orofacial cleft (14). The branchial arches, which appear on the 4th week of gestation, fuse in the midline on the 7th week, and they form the face, which consists of neural crest cells and mesoderm. The neural crest is differentiated earlier in embryogenesis in comparison to the neural plate. It plays a pivotal role in the development of many structures, such as the heart, the middle ear, the connective tissue around the eye, the iris and the pupils, the skin, the adrenal medulla, several cranial nerves, and the celiac ganglia. Anomalies of neural crest cell differentiation can lead to a broad spectrum of malformations. Neural crest cell dysfunction may lead to orofacial cleft formation, congenital heart anomalies (15), Hirschsprung's disease (16), developmental disorders of the middle ear (17), abnormal pigmentation of the skin (18), tooth malformations, and colobomata (19, 20). The middle and more cranial part of the face is formed through cell proliferation of neural crest cells and ectoderm, and the rest of the face is from the neural crest cells. Developmental deviations of neural crest cells are responsible for facial deformations (21) but neither for the extremities nor the urogenital, kidneys, airway (positional) deviations, and the abdominal wall.

Almost 9% of the children in this study had a syndrome. In a retrospective study presented by Lilius, 5 out of 113 (4.4%) patients with BCLP had a chromosomal anomaly. Lilius's study showed data collected through birth certificates, mothers' reports post-partum, and all the data available on the records of the cleft team of Finland. There were available records of an 11-year follow-up of these patients (10). Associated chromosomal disorders and syndromes have been reported more often in children with cleft lip or palate or unilateral cleft lip and palate (10). In the study of Stoll *et al.*, 11% of their patients with orofacial cleft had an associated syndrome or a chromosomal disorder (8). The most common associated syndrome was trisomy 13, observed in 2.4% of their patients (8). Milerad *et al.* reported in their prospective study a syndrome or chromosomal deviation in 5.4% of their patients with an orofacial cleft. In this study, van der Woude syndrome was the most commonly observed, with

an incident of 0.6% (3). In a retrospective database in Hungary, where only live births were included, 1% of patients with orofacial cleft had an associated syndrome or chromosomal anomaly. Trisomy 13 was the syndrome most often observed (22). The increased incidence of reported syndromes or associated malformations in children with BCLP of this study compared to other studies might be because new diagnostic tools became available during the years. Additionally, the awareness of associated anomalies in children with CLP in contrast to the general population increased.

This study is a retrospective study; therefore, there is a specific limitation. Specific congenital malformations of these patients may not have been registered. Therefore, the actual incidence of congenital malformation in this group of patients may be higher. In this study, we present congenital malformations, which have been registered for the first three years of the life of patients with cBCLP. The incidence of the associated congenital malformations presented may be underestimated.

Conclusions

Children with a cBCLP often have other associated congenital malformations. Specific syndromes have also been diagnosed in 9% (out of 133) of this group of children. Therefore, pediatricians should pay particular attention to this group of children for associated congenital malformations or syndromes.

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Table 1. Congenital malformations diagnosed in 133 children with complete bilateral cleft lip and palate

Congenital malformations diagnosed during the three years of follow up			Number of patients (n=)	Percentage (n/133)
			44	33.1%
Heart	Total		**11**	**8.3%**
		Ventricular septal defect	4	3.0%
		Aortic coarctation	3	2.3%
		Atrium septum defect type I and persistent ductus arteriosus	2	1.5%
		Tetralogy of Fallot	2	1.5%
Respiratory system	Total		**2**	**1.5%**
		Laryngomalacia	1	0.8%
		Tracheoesophageal fistula	1	0.8%
Gastrointestinal	Total		**2**	**1.5%**
Urogenital	Total		**14**	**10.5%**
		Cryptorchidism	7	5.3%
		Other abnormalities of male genitalia	4	3.0%
Central nervous system	Total		**5**	**3.8%**
		Semilobar holoprosencephaly, hypophyseal dysfunction	1	0.8%
		Cerebellum and vermis hypoplasia	1	0.8%
		Tetraventricular hydrocephalus, cisterna magna cysts	1	0.8%
		Agenesis of the corpus callosum, diabetes insipidus	1	0.8%
		Atrophy of the frontal brain lobe (cerebrum)	1	0.8%
Skin disorders	Total		**3**	**2.3%**
		Scalp, hair, and hypoplastic nipples	1	0.8%
Extremities	Total		**12**	**9.0%**
		Deformations / digital agenesis	9	6.8%
Developmental disorders	Total		**7**	**5.3%**
		Psychomotor retardation	7	5.3%
Facial	Total		**19**	**14.3%**
		Hypertelorism	6	4.5%
		Shape deviation / big skull	4	3.0%
		Other deviations not described	5	3.8%
		Micrognathia / retrognatia	3	2.3%
Neck, nose, ear, and eyes	Total		**8**	**6.0%**
		Coloboma	5	3.8%
Other	Total		**17**	**12.8%**
Dental	Total		**2**	**1.5%**
		Tooth agenesis		0.8%
		Tooth shape deviations	1	0.8%

Table 2. Syndromes and chromosomal disorders in 133 children with complete BCLP

Syndrome	Number of patients (n=)	Percentage (n/133)
EEC syndrome	2	1.5%
Amniotic band syndrome	2	1.5%
CHARGE syndrome	2	1.5%
BBB- syndrome	1	0.8%
Down syndrome	1	0.8%
ADAM syndrome	1	0.8%
Nager syndrome	1	0.8%
13q-syndrome	1	0.8%
Brachio-oculo-facial syndrome	1	0.8%
Total	12	9.0%

BCLP: bilateral cleft lip and palate
EEC syndrome: "Ectodermal dysplasia, Ectrodactyly, Clefting" syndrome
CHARGE syndrome: "Coloboma, Heart anomaly, Choanal atresia, Retardation, Genital and Ear anomalies" syndrome
BBB- syndrome: the first letters of the last names of the families first diagnosed with this disorder (hypertelorism, oesophageal malformation, and hypospadias)
ADAM syndrome: "Amniotic Deformity, Adhesions, Mutilations" syndrome

Table 3. Results of this study in comparison to the published data of Lilius. (10)

Congenital malformations	Results of this study (n = 133)	Results of Lilius 1992 (n = 113)
Total prevalence	**33.1%**	43.4%
Heart	**8.3%**	15.9%
Gastro-intestinal	**1.5%**	8.8%
Urogenital	**10.5%**	8.0%
Cervical vertebra	**0%**	-
Central nervous system	**3.8%**	8.0%
Extremities	**9.0%**	33.6%
Developmental deviations	**5.3%**	8.0%
Facial	**14.3%**	13.3%
ENT and eye	**6.0%**	10.6%
Other	**12.8%**	0.1%
Chromosomal anomalies	**9.0%**	4.4%

ENT: ear, nose, and throat

2.2 Tooth agenesis patterns in unilateral cleft lip and palate in humans

Bartzela TN, Carels CEL, Bronkhorst EM, Kuijpers-Jagtman AM.
Arch Oral Biol. 2013;58(6):596-602. doi: 10.1016/j.archoralbio.2012.12.007

Summary

Factors possibly contributing to tooth agenesis (TA) inside or outside the cleft area are disturbances during embryogenesis and/or possible iatrogenic injuries during surgical procedures. This study described TA patterns of dentition and their prevalence in a group of complete unilateral cleft lip and palate (cUCLP) patients using a numeric coding system, the Tooth Agenesis Code (TAC).

Design: Panoramic radiographs of 115 non-syndromic patients (78 males and 37 females) with cUCLP (85 patients had a left-sided cleft and 30 right-sided) from the Cleft Palate-Craniofacial Unit in Nijmegen (The Netherlands) were evaluated. Third molars were excluded from the evaluation. Pre-operative records confirmed the diagnosis of cUCLP. Patients with Simonart`s band were not included (N=18). Only patients with Caucasian ethnic background were evaluated. No other obvious congenital malformations or mental retardation were associated with the cleft lip and palate (CLP). According to the center's routine procedure, at least three panoramic radiographs were available, taken around 9, 11, and 14 yrs. The treatment was performed according to the same protocol.

Results: Agenesis of at least one tooth was observed in 48.7% of the patients and outside the cleft in 20.9%. One to three were missing teeth per patient, whereas 51.3% had no TA. The lateral incisor of the maxillary cleft quadrant was the tooth most frequently missing (39.1%), followed by the maxillary lateral incisor (8.7%) and the mandibular second premolar (7.8%) in the non-cleft quadrants. Thirteen different TA patterns of patients with cUCLP were found in this study. Six of the identified patterns were observed in only one patient. TA was significantly higher on the cleft quadrant of the maxilla ($p = 0.020$). The cleft's sidedness was related to the TA. Therefore, on the right side were fewer missing teeth compared to the left ($p = 0.18$).

Conclusion: TA was observed in a higher prevalence in patients with cUCLP, inside and even outside the cleft region, compared with the non-cleft patients. Thirteen different TA patterns were observed, and six of them were unique. Maxillary and/or maxillary and mandibular second and/or first premolars were involved in all TA patterns. Both the prevalence of orofacial clefting and hypodontia are more frequently observed on the left side.

2.3 Apical root resorption after orthodontic treatment in patients with unilateral cleft lip and palate

Bartzela TN, Mang de la Rosa MR, Wolf K, Schmidt A, Opitz C.
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Summary

External apical root resorption (EARR) is a common adverse effect observed during orthodontic treatment. The high inter-individual variability that is observed indicates a multifactorial etiology. The risk factors that are responsible for the severe EARR remain unclear. Patient's age, endocrinological disorders (e.g., hypothyroidism or reduced estrogen levels, metabolic factors like prostaglandins, and nutritional deficiency (in Ca or Vitamin D) have been associated with EARR. Other factors, such as fluoride, thyroxine, steroids, and the administration of PGE2 together with thyroxine, may have a protective effect over the EARR, as has been proved in animal studies.

Patient-related intraoral predisposing factors to EARR are abnormal root morphology, and dental trauma, especially in association with preexisting EARR. Contrarily, endodontically treated teeth and teeth with incomplete root formation develop less commonly EARR.

The orthodontist has a challenging role in patients with craniofacial anomalies, such as cleft lip and palate (CLP). Patients with complete unilateral cleft and palate (cUCLP) usually need a complicated and long-lasting orthodontic treatment due to the constricted and asymmetric maxillary arches, deviated premaxilla, and bone and soft tissue deficiency in the cleft area. Often the teeth located in the cleft's vicinity are impacted, ectopic, missing, or rotated. Therefore, it is crucial to evaluate the incidence and predisposing factors that lead to EARRs in these patients.

This retrospective longitudinal study aimed to evaluate the incidence of EARR in the anterior teeth on the affected and non-affected sides of patients with cUCLP. Furthermore, to estimate to what degree patients' and treatment variables during the orthodontic treatment influence the development of EARR in these teeth.

Material and Methods: In this study, orthopantomograms (OPGs) of forty-one patients with cUCLP taken before (T2) treatment with multibracket orthodontic appliances (MBA) and

periapical radiographs (PAs) of the anterior maxillary teeth taken at the end (T3) of orthodontic treatment were assessed for EARR.

Results: At T3, the incidence of EARR was considerably higher (97.6%) than at T2 (51.2%). EARRs preexisting before the orthodontic treatment and abnormal root morphology are considered predisposing factors. Central incisors and canines on the cleft side have a significantly higher score of EARR ($p < 0.01$, $p < 0.05$, respectively) compared to the teeth on the non-cleft side.

Conclusions: Patients with cUCLP treated with MBA during the orthodontic treatment have a higher incidence of EARR on the maxillary central incisors of the cleft side. Severe EARR is relatively rare.

Clinical relevance: Most patients with CLP need a challenging and long-term orthodontic treatment with an MBA. Therefore, it is essential to identify the predisposing factors related to the teeth' particular anatomical features and the bone adjacent to the cleft area. Abnormal root morphology was not among the risk factors for EARRs. The EARRs during orthodontic treatment are mostly less than two millimeters and therefore are of no clinical relevance.

2.4 Photographs of dental casts or digital models: Rating dental arch relationships in bilateral cleft lip and palate

Leenarts CMR, Bartzela TN, Bronkhorst EM, et al.
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Summary

Yardsticks have been developed to evaluate dental arch relations in patients with CLP, representing a proxy of the underlying skeletal structures. The use of plaster casts for inter-center studies is complicated because of the difficulty of carrying them to other centers. Therefore, we aimed to investigate the reliability of using digital models or photographs of dental casts alternatively to plaster casts for assessing dental arch relationships in children with complete bilateral cleft lip and palate (cBCLP).

Plaster dental casts, digital models (3D scans), and photographs of twenty children (n=20) with cBCLP were evaluated. All three record formats were available at 6, 9, and 12 years of age. Patients with syndromes or Simonart's bands were excluded from the study. Four observers scored the casts and pictures using the BCLP yardstick. The interrater weighted kappa scores were from 0.672 to 0.934. No significant differences in the BCLP yardstick scores were observed between the three forms. The observers' intra-rater weighted kappa for the three different formats was between 0.692 and 0.885. It is concluded that digital models and photographs of dental casts can be used for rating dental arch relationships in patients with cBCLP.

These formats are a reliable alternative for BCLP yardstick assessments on conventional plaster casts. The photographs and digital models can replace the plaster models for the rating of the dental arch relationship in patients with cBCLP with the use of the BCLP yardstick at the age of 6 (range 5.0 to 6.6), 9 (range: 7.11 to 10.1), and 12 years (range 10.10 to 13.8). The overjet and crossbites were more difficult to be assessed in photographs than in dental casts or digital models. This difficulty, though, did not affect the final scoring outcome. It was found that the plaster models were rated roughly 0.1 higher than the two other formats.

The statistical analysis on the reproducibility of the three methods was found to be satisfactory. Therefore, it can be concluded that intercenter treatment outcome comparisons of patients with CLP can be performed via data transfer through CD or e-mails, making such studies easier to conduct.

2.5 Clinical characterization of 266 patients and family members with cleft lip and/or palate with associated malformations and syndromes

Bartzela T, Theuerkauf B, Reichardt E, Spielmann M, Opitz C

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Summary

Orofacial clefts (OFC) have a significant medical and social impact on the affected individuals and their families. The family recurrence of nsCL/P varies depending on the type and severity of the cleft. However, almost 70% of the OFC are nonsyndromic (nsCL/P), and 30% are associated with a syndrome or congenital malformations. Syndromic CL/P (sCL/P) and nsCL/P patients have distinct etiopathogenesis. The severity of cleft is playing a role in the association with a malformation or syndrome.

Consequently, patients with bilateral CL/P (BCL/P) are more frequently affected by congenital malformations or syndromes. Patients with cleft palate only (CPO) are more often associated with a syndrome. Almost 600 syndromes are related to OFC, and 400 have been associated with CPO.

Objectives: To identify the clinical phenotypes of patients and family members with Cleft Lip and/or Palate (CL/P) and associated congenital malformations or syndromes and suggest a possible mode of inheritance.

Materials and Methods: In a retrospective observational study, patients with CL/P and their family members have been examined intra- and extra-orally. Their medical and family history was registered.

Results: In total, 266 index patients, 1,257 family members, and 43 pedigrees were investigated. According to the cleft type, the patients' allocation was: 57.9% with CLP, 25.2% with cleft palate (CPO), and 12.8% with cleft lip with/without alveolus (CL/A). Almost one-third of the patients (in total, 74) had an associated malformation, and 24 (9.2%) had a syndrome. The skeletal and cardiovascular malformations were the most commonly observed. Pierre Robin sequence and van der Woude syndrome were the most common syndromes associated with a cleft. In most of the syndromes (19/24), the cleft phenotype was the CPO. An average of 2.45 affected members were registered.

Conclusion: The etiopathogenesis of CL/P is challenging to be unraveled because of the phenotypic variability among individuals and family members.

Clinical relevance: The overall prevalence of patients with CL/P and their family members with an associated malformation or syndrome points out the need for interdisciplinary treatment, early diagnosis, and long-term planning. A particular mode of inheritance could not be identified in these pedigrees. A further molecular investigation is required to elucidate the etiopathogenesis of these phenotypes.

3. Discussion

3.1 Results of the studies

3.1.1 [Associated congenital malformations in patients with complete bilateral cleft lip and palate]

Data were collected from patients with complete bilateral cleft lip and palate (cBCLP) born before 2003. The patients were treated from birth in the cleft center of the Radboud University of Nijmegen. Patients' associated anomalies and syndromes were registered. The first documentation for associated malformations and syndromes was performed when the children were three years of age. The patients were followed up for complications or additional congenital malformations during their follow-up visits to the center.

We reported the congenital malformations of patients with cBCLP during the first three years of their life. According to the Dutch registry, most congenital malformations have been diagnosed until the third year of patients' life (Rozendaal et al., 2012). An additional 2-3 % of malformations were diagnosed at four years of age (van der Veen et al., 2006). Moreover, at the age of 12, an extra 5% of these patients were diagnosed with an associated malformation (van der Veen et al., 2006).

In our study with cBCLP, 64 (48%) were diagnosed with an associated malformation (Kouwenberg et al., 2010). Mainly, the malformations diagnosed were on the face, extremities, urogenital and cardiovascular systems. These findings are in mind with other studies (Lilius, 1992). Patients with CPO have more often associated with malformations (46.7%) than the different types of clefts (CLP: 36.8% and CLO: 13.6%) (Stoll, 2000).

For the first time, our study reported dysmaturity and failure to thrive (in 12.8% of these patients) and cervical vertebral malformations in patients with BCLP (Kouwenberg et al., 2010).

Furthermore, more males with cBCLP were observed in this group of patients than females (male/female ratio 2.2), following previously published data (Al Omari and Al-Omari, 2004). Nevertheless, the sex predisposition in patients with BCLP with an associated syndrome or chromosomal disorder is presented for the first time. In these patients, a predominance of females has been observed. The male/female ratio in patients with cBCLP with associated

congenital anomalies or syndromes was 0.71 (p = 0.05). Additionally, more data are required to elucidate sex preference in patients with BCLP and associated anomalies and syndromes. This study's results have pointed to an increased incidence of multiple congenital anomalies in patients with BCLP. Therefore, the cleft team should conduct a critical medical assessment, especially for patients with BCLP.

In this study, 9% (12 out of 133) of the children had an associated syndrome. In other studies, 5.4% of the patients reported a syndrome or chromosomal anomalies (Milerad et al., 1997). The most common syndrome registered in patients with OFC was trisomy 13, with a prevalence of 2.4% (Stoll, 2000), which other authors also reported (Sárközi et al., 2005).

Another study reported that van der Woude syndrome is the most common syndrome observed in patients with CLP, with an incidence of 0.6% (Milerad et al., 1997). The incidence of associated syndromes or malformations in children with BCLP presented in this study, compared to other investigations, is most probably related to the increasing awareness over the years and modern diagnostic tools enable the early identification and improved prognosis of these children (Bartzela, 2019).

3.1.2 Tooth agenesis patterns in patients with unilateral cleft lip and palate in humans

The tooth agenesis (TA) patterns in patients with complete unilateral cleft lip and palate (cUCLP) treated from birth in Radboud university medical center (Radboudumc), Nijmegen, the Netherlands, were evaluated. A numeric code, the Tooth Agenesis Code (TAC), was used to present TA patterns of permanent dentition (van Wijk and Tan, 2006). TA is a common finding of OFC patients and may be associated with developmental defects during embryogenesis and surgical procedure in the cleft region (Lekkas et al., 2000). TA is more frequently observed in the maxilla (Hellquist et al., 1979), especially in the union of the median nasal and the maxillary processes, resulting in agenesis of the lateral incisors (Hovorakova et al., 2006). The maxillary or/and mandibular premolars were identified in all agenesis patterns of this study. The most common symmetric TA patterns were the maxillary lateral incisors (5.2%) and the mandibular second premolars (0.9%) (Ranta, 1986). In a big cohort of patients with cBCLP, the lateral incisors in the cleft quadrant and the maxillary and mandibular second premolars were the most commonly missing teeth (Bartzela et al., 2010b). Almost 50% of the patients included in this study had at least one missing tooth (Bartzela et al., 2013). The TA outside the cleft area in cUCLP varies from 27–28% (Brattstrom et al., 1989; Dewinter et al.,

2003) to 48.8% (Ranta, 1986). In this cUCLP cohort, only 19.1% of the patients had TA outside the cleft area. The high variability of TA patterns outside the cleft area could be ascribed to ethnic and genetic diversity.

TA in areas outside the cleft accentuates a common genetic pathway of tooth development and palatogenesis (Eerens et al., 2001).

Studies on siblings and parents of patients with CL/P indicate a higher prevalence of TA than in non-affected individuals (Howe et al., 2015). Studies on twins and family members underline the contribution of shared environmental and genetic factors (Mansilla et al., 2005).

Patients with a cleft on the right side were less affected by TA than patients with a left-sided cleft. The cleft-sidedness and TA have been observed in patients with syndromes and associated congenital affected organs on the left side, like congenital heart defects. Occulo-facio-cardio-dental (OFCD) syndrome is associated with pathogenic variants of the *BCOR*-gene. The *BCOR*-gene contributes to morphogenic organ development by defining the sidedness patterning (Hilton et al., 2007; Hilton et al., 2009). If a correlation between TA, cleft-sidedness, and heart defects is identified, we may gain insight into the left-sided clefting etiology.

3.1.3 Apical root resorption after orthodontic treatment in patients with unilateral cleft lip and palate

External apical root resorption (EARR) is a common side-effect of orthodontic treatment. The maxillary incisors have been indicated as the most affected teeth (Barros et al., 2017). In this study, we examined if the anterior teeth of patients with cUCLP have a higher risk of developing EARRs on the cleft side during an orthodontic treatment than the contralateral, non-affected side. Moreover, to evaluate the effect of different factors related to the multibracket (MBA) orthodontic treatment in developing EARRs in these teeth.

Rigid inclusion criteria were used to control follow-up bias and factors that may affect the study's outcome. Teeth with incomplete root formation or size and shape deviation were excluded from the evaluation. The laterals and canines, especially on the cleft side, were the teeth included. Nevertheless, a significant sample size (n = 80) of the central incisors on the cleft and non-cleft sides has been evaluated.

The incidence of patients with EARR in this study was 97.6%. The central incisors (80.0%) and the lateral (69.9%) were the most frequently afflicted teeth. Most of the evaluated teeth (88.1

%) had a resorption score of no clinical relevance (score 1 or 2) (Roscoe et al., 2015). Only 1% of the teeth had an EARR of clinical significance (score 4). The EARR of low occurrence during the OT has been described in different clinical studies (Mirabella and Årtun, 1995a).

The OT duration with the MBA was not identified as a factor predisposing EARR in this study (p = 0.515). In the scientific literature, some studies confirm that the orthodontic treatment duration is a factor predisposing to EARR (Fox, 2005; Årtun et al., 2005), while others contradict it (Mirabella and Årtun, 1995b).

The maxillary lateral incisors are sensitive to developmental anomalies, leading to size and shape deviations. Therefore, these teeth are often extracted, and space is preserved for prosthodontic rehabilitation or is closed using MBA.

Teeth on the cleft side were more predisposed to EARR than the teeth on the non-cleft side, most probably because these are severely malpositioned.

The type of OTM may affect EARR. In our study, the lateral incisors' crown sagittal displacement had a significant effect on the EARR.

In other studies, though, it has been reported an association of the EARR with the total sagittal apical displacement towards the cortical bone (Baumrind et al., 1996), while other studies contradict these conclusions (Mirabella and Årtun, 1995b). Increased EARR is related to the amount of root movement and not contact with the cortical bone (Mirabella and Årtun, 1995b). Some studies confirm these findings, proving that the dentoalveolar bone's morphology and thickness are not factors that predispose EARR (Otis et al., 2004).

Preexisting EARRs (Jiang et al., 2010) and initial atypical root form (Mirabella and Årtun, 1995b) are predisposing factors for EARR during orthodontic treatment. The preexisting EARR, as a predisposing factor, was also confirmed in this group of patients.

In our study, the use of intermaxillary elastics was not a predisposing factor for EARR. Some studies agree with our findings (Årtun et al., 2009; Motokawa et al., 2012), and others consider intermaxillary elastics as a risk factor for EARR (Mirabella and Årtun, 1995b). In this group of patients, oral habits after the age of seven were not correlated to EARR. There is also a controversy in the research findings regarding an association between severe oral habits and EARR. Some researchers consider intense habits as predisposing factors (Odenrick and Brattström, 1985) while others contradict it (Nanekrungsan et al., 2012b).

3.1.4 Photographs of dental casts or digital models: rating dental arch relationships in bilateral cleft lip and palate patients

The BCLP yardstick is a relatively new tool for treating patients with cBCLP (Ozawa et al., 2011). This yardstick evaluates the sagittal relationship of the maxillary and mandibular dental base. This yardstick considers the relationship between the dental arches and the patients' craniofacial development (Bartzela et al., 2010a). A measurement system that has been used for dental outcomes in patients with cleft lip and palate (CLP) patients is the Huddart/Bodenham (HB) system. This system presents the sagittal and transverse relationship of the dental arches. We have compared the two scoring systems (HB-system with the BCLP yardstick) in patients with cBCLP in three different age groups (Bartzela et al., 2011). The BCLP yardstick has been used for intercenter evaluation of patients with cBCLP (Bartzela et al., 2010a).

This yardstick is a subjective tool because it represents the dental arch relationship's orthodontic management potential. Therefore, a certain degree of orthodontic experience and good calibration is required before use (Bartzela et al., 2010a).
The yardstick was assessed in different forms of dental casts, plaster, digital, and 2D photographs. The plaster casts were the reference models. The BCLP yardstick defines scores between one and five, of which one represents the best treatment outcome and five the worst.

During the evaluation session, the raters observed that it was more challenging to assess the crossbites or the overjet in photographs than in cast and digital models. However, this limitation was not entirely reflected in the scorings. The dental casts and digital models offered the added option to rotate the models in all directions, thus facilitating the assessment of anteroposterior and transverse relationships. Our findings showed that photographs and digital models could be used to evaluate dental arch relationships in patients with cBCLP with the BCLP yardstick at the age of 6, 9, and 12 years. Photographs and digital models can replace the conventional dental casts for allocating the models in a score based on the BCLP yardstick. The scores based on different formats showed only minor differences. The plaster models had approximately 0.1 higher scores. Since we do not have a gold standard, we can not say which dental arch form performs better. The statistics giving information on the reproducibility of the three formats were very comparable and of satisfactory level. However, the observer mentioned the difficulty of evaluating the patients' overjet or crossbite on the dental models' 2D

photographs. This limitation, though, was not reflected in the scoring outcome. The digital model offers the option to rotate them, facilitating the final assessment.

This study showed that the plaster models could be replaced by photographs or 3D models copied in CD or online transfer for intercenter comparisons or remote clinical evaluations for CLP patients. The images could be placed on a password-protected website to fulfill the strict patients' data protection rights, permitting the clinician or rater to make the scoring from his computer. According to the "Good Practice Archives," archives should be designed for general sharing, including learning modules for new users (Nollet et al., 2004).

3.1.5 Clinical characterization of 266 patients and family members with cleft lip and/or palate with associated malformations and syndromes

Patients with CL/P examined at Charité - Universitätsmedizin Berlin between 28.01.1999 and 25.05.2000 were included in the study. Patients were routinely followed up once a year based on their day of birth. Only 50% of the patients examined in the first semester of the year accepted to participate. Nevertheless, 1257 family members and 42 pedigrees were included. The clinical characteristics of these patients and their family members are presented. The constructed pedigrees were used to determine the inheritance patterns of these patients.

In this study, patients with CLP comprise the biggest group of index patients *(*38.2%). 65.7% of the index patients had UCL/P (Table 1). Left-sided CL/P is the most common type of clefts, observed in 2/3 of all UCL/P patients (Yi et al., 1999). Patients with CPO (25.2 %) and BCLP (19.5%) were also registered (Table 1).

Males were more than females in CL/P groups (Table 1). Nonetheless, the incidence of patients with CPO is consistently higher in females (Matthews et al., 2007). OFC counts only for 0.4% (only one patient) in this study, which agrees with other reports (0.43-0.73%) (Watanabe et al., 2019).

The associated anomalies were related to the severity of the cleft (Group, 2011). Patients with CPO have a higher incidence of malformations compared to other types of clefts (Stoll, 2000),(Watanabe et al., 2019), which is a common finding in this study.

The individuals with BCLP are more commonly affected with malformations and syndromes than the UCL/P patients (Kouwenberg M, 2010). Females were more likely to have a severe

type of cleft and associated congenital abnormalities ranging up to 62% (Sivertsen et al., 2008a).

Following this study's finding, the skeletal system and extremities' malformations were observed in almost 1/3 of the patients (29.7%) (Lilius, 1992). Cardiovascular anomalies in patients CL/P varied from 4.3% (Jensen et al., 1988) to 63.4% (Shprintzen et al., 1985). Associated anomalies of the CNS (14.3%), the urogenital system (Stoll, 2000), and facial abnormalities (13.0%) (Lilius, 1992) are commonly reported.

Nearly 70% of the patients with a cleft are non-syndromic (Stanier and Moore, 2004), and 30% are syndromic. Almost 600 syndromes have been identified. Most of these syndromes are associated with CPO (Dixon et al., 2011),(Leslie and Marazita, 2013).

The most common syndromes observed in this group of patients were the PRS (9 patients) and the VWS (4 patients). Other common associated syndromes with a CL/P are the 22q11.2 deletion, the Kalman syndrome, Ectodermal dysplasia, ectrodactyly cleft syndrome (EEC3), and the Kabuki syndrome (Bartzela et al., 2017).

The high variability of the associated anomalies in patients with CL/P is related to the examination criteria, type of cleft, patient age at the diagnosis, ethnic background, and malformations reported (Tolarova and Cervenka, 1998).

Many associated anomalies or syndromes are identified only later on in life (e.g., lip pits in the VWS) due to developmental variability among individuals or unremarkable family history. It has been reported that one out of the six associated anomalies has been diagnosed beyond the first year of life (Bower et al., 2010). Dental anomalies are mainly excluded because they are positively correlated to clefts (Phan et al., 2016).

The examination of patients with CL/P and their pedigrees demonstrated a high rate of familial recurrence. The pathogenesis genetic and environmental triggers of syndromic (sCL/P) can be the same as those of nonsyndromic (nsCL/P) (Wang et al., 2016).

The clinical diagnosis in patients with known family history is challenging. The cleft team members should be suspicious of an associated malformation. Therefore, early genetic counseling and regular follow-up appointments should be considered in patients with OFC (Rozendaal et al., 2012).

3.2 Strengths and weaknesses of the studies and methodological considerations

In this thesis, the collected data were from the files of Charité - Universitätsmedizin Berlin, Germany, and Radboud University Medical centre, Nijmegen, the Netherlands. As the data presented are retrospective, we must be aware of selection and follow-up bias; therefore, we set a strict protocol for the data acquisition.

There is a lack of controlled studies on patients with cBCLP for many reasons. The incidence of BCLP is very low. Only 7% of cleft patients have a cBCLP phenotype (Sivertsen et al., 2008a). Most European Cleft Centers (76%) have a caseload of 40 or fewer CLP patients. When we conducted our studies, the published retrospective studies did not exceed 57 patients with BCLP patients (Semb, 1991).

Publications on patients with cBCLP and associated syndromes or malformations are even more scarce. We are presenting the results of 102 patients with cBCLP and associated malformations and syndromes (Kouwenberg et al., 2010). Our results are consistent with previously reported data on associated congenital anomalies in patients with BCLP. Female predominance in syndromes has not been previously reported.

One of the studies included in the thesis describes patients' phenotypic variability with CL/P and their family members. Data of 266 index patients and 1257 family members participated in the study.

The results are based on recorded information; therefore, associated malformations and syndromes of these patients may be missing or underestimated.

Moreover, a diagnosis of the congenital disorder in patients without a family history of associated malformations or syndromes may not be diagnosed until the 4^{th} or 5^{th} year of life (van der Veen et al., 2006). The reason is that either the problem is not apparent yet or is mild due to developmental variations.

For example, the CLP team often diagnoses velocardiofacial syndrome only when the patients have speech and learning difficulties. Furthermore, mitral valve prolapse is considered an autosomal transmitted congenital condition, but the prolapse may not be developed in many cases. Other congenital heart problems may fade gradually as the patients grow (Barbosa et al., 2003).

The incidence of congenital anomalies in patients with CLP can include sampling bias. Many authors report infants from a medical unit and others from whole geographical areas. Selection bias also should be encountered since only survivors are registered without including stillbirths. Unfortunately, often the sample's restrictions are not clearly defined.

It is suggested that each group of patients with CLO, CPO, CLP non-syndromic, syndromic, and patients with associated anomalies, live births, stillbirths, and abortions should be presented separately (Vanderas, 1987).

Associated malformations are grouped as major (functional implications) and minor (aesthetic impact) (Monlleó et al., 2015). Dental anomalies are mainly excluded because they are positively correlated to clefts (Phan et al., 2016).

The increased infant mortality rate (IMR) in patients with CL/P is related to the associated malformations or syndromes (van Nunen et al., 2014).

Associated malformations and syndromes have increased reported incidence over the years due to the new diagnostic tools and the raised awareness of the multidisciplinary teams.

More research is required to identify the risk factors of OFC in family members in different populations and ethnic groups (Vanderas, 1987).

Furthermore, detailed phenotyping of these patients and unraveling the pathogenic process's primary mechanism would help us identify preventive and therapeutic instruments (Bartzela et al., 2017).

Antioxidants may prevent craniofacial malformations by reducing cell death in the neuroepithelium, as shown in Tcof1(+/−) mice (Sakai et al., 2016).

Epidemiological data and clinical studies are required to identify prenatal teratogenic exposures, epigenetic factors, bias, and chance contributing to craniofacial anomalies (Bartzela et al., 2017).

Epigenetic factors (Takahashi et al., 2018) or genes that contribute to organ development's laterality may define the laterality of the cleft, e.g., the BCOR-gene in the Occulo-facio-cardio-dental syndrome (OFCD) (Hilton et al., 2007; Hilton et al., 2009).

Moreover, variants in *PBX1, PBX2,* and *TP63* have contributed to nsCL/P's pathogenesis (Maili et al., 2020) and in *TBX22, P63,* and *FGFR1* to sCL/P (Zucchero et al., 2004). The *X-chromosomal recessive* form has been identified in families with a cleft of the secondary palate (overt or submucous), and it is often associated with ankyloglossia (Pauws et al., 2009).

A gene-specific mutation or the associated *IRF6* haplotype could raise the recurrent familial risk from the practical value of 3 to 5 percent, making critical genetic counseling for subsequent pregnancy (Zucchero et al., 2004). Therefore, molecular prevention counseling in families at risk and studies to identify exogenous risk factors are mandatory. Identifying

contributing factors may lead to primary preventive interventions, improved genetic counseling, and future preventive action.

Tooth agenesis patterns in patients with cUCLP (Bartzela et al., 2013) are included in this thesis. A high prevalence of tooth agenesis is observed in the cleft and outside the cleft region in patients with cUCLP compared to the general population. We presented the cleft and non-cleft sides separately because differences in TA were expected. To our knowledge, the study on patients with UCLP and tooth agenesis was the second to present patterns of tooth agenesis in the whole mouth and not restricted only to the maxilla or the cleft area (Bartzela et al., 2013).

Thirteen different patterns in the whole mouth were observed, of which six were unique patterns (observed only in one patient). Premolars and/or second incisors were involved in all agenesis patterns. Both the prevalence of orofacial clefting and hypodontia are more frequently observed on the left side, confirming other studies' results.

The tooth length was measured along the pulp canal up to the incisal edge. Other investigators have used the cementoenamel junction for their evaluation. The method we chose to use was more accurate, as it has been proved by other investigators (Brice et al., 1991; Mirabella and Årtun, 1995a).

The orthodontic treatment, the anatomic variability in the cleft area, or the intrinsic patients' factors may trigger the EARR. Longitudinal retrospective studies may obscure the factors that may predispose to EARR. It is challenging to assess (Bartzela et al., 2020) the multifactorial parameters' specific role that activates the EARR in a longitudinal retrospective study. Long-term prospective multicenter studies may elucidate patients' and treatment variables relevant to EARR (Bartzela et al., 2020). Moreover, EARRs were assessed on two-dimensional images (OPGs and periapical (PA) radiographs). Therefore, lesions on the palatal or labial surface are not detectable. Data collected from CBCT showed more accurate results for EEAR (Deng et al., 2018). Many confounding variables may affect the recognition of factors related to EEAR. Prospective clinical trials may help identify the predisposing factors and define treatment protocols to eliminate EARR (Deng et al., 2018).

No significant differences were found for the BCLP yardstick scores between the three formats and the four raters (Leenarts et al., 2012). Digital models and photographs of dental casts can be used for rating dental arch relationships in patients with cBCLP. These formats can replace the conventional plaster casts for intercenter treatment outcome evaluation.

Nevertheless, facial growth and primary surgery in different centers can be appreciated only when cranial growth patterns are known. Therefore, the cranial base's morphology should be evaluated as determining factor in the dental and skeletal relationship.

Evidence-based medicine is necessary to give insight to the patients and the interdisciplinary team for health care improvement.

Systematic reviews (SR) or meta-analyses (MA) provide the strongest evidence for practice decisions. Randomized controlled trial (RCT) provides, in second place, evidence for the examined interventions (McPeek et al., 1989). Therefore, as included in this thesis, case series studies should be replaced by prospective clinical trials.

Nevertheless, there are many difficulties in performing trials, especially in patients with OFC. Therefore, only a few RCTs exist and often demonstrate limited evidence.

Patients with OFC need treatment from birth until the end of craniofacial growth (Bartzela, 2019), and there is a need to determine the impact and outcome of each intervention. Furthermore, long-term monitoring is required for the registration of scarce or late outcomes. Moreover, even if the surgical procedures are the same, there are many factors like minor surgical details, surgeons' learning curve, and different levels of training of the surgical team (Bartzela, 2011) that make the comparisons challenging. The various teams are using different scales for rating the severity of the cleft, making the outcome assessments and intercenter comparisons difficult. For these reasons, the treatment interventions and surgical procedures performed in patients with OFC are based on retrospective studies, surgeons' choice, and in some cases, even the burden of care (Hall et al., 2009).

Conclusively, we could say the alternative perspective designs could be used for outcome evaluation in patients with OFC, such as cohorts with cross-sections over the observational time. Suppose the most critical confounders are measured, and satisfactory outcomes are used uniformly. In that case, advanced statistical techniques can be employed to give valuable information for improving the treatment outcome of individuals with congenital craniofacial anomalies.

3.3 Clinical implications

In this thesis, the clinical phenotypes of patients with CLP are presented (Bartzela et al., 2021). Associated syndromes and malformations of these patients have also been reported (Bartzela et al., 2021) to improve communication and interaction of the specialties involved in treating these

patients. Knowledge gaps and differences in diagnostic definitions, medical records, and OFCs' treatment make mandatory multidisciplinary collaboration on a national and international level. Identifying craniofacial characteristics and associated malformations can lead to an early diagnosis and recognition of genetic risk factors among non-affected individuals (Richmond et al., 2018). The extensive phenotypic variability, even among relatives, often makes an early diagnosis challenging (Bartzela et al., 2021). Patients with unknown family history, especially an early diagnosis, are even more complicated.

Genetic and environmental factors contribute between the 5th and 8th gestational week to the etiopathogenesis of these syndromes. Prenatal diagnosis is essential for better genetic counseling, postpartum management, treatment planning, and parenthood preparation (McDonald-McGinn et al., 2015). Preventive intervention from early pregnancy, implemented by agents such as antioxidants (Sakai et al., 2016), may help prevent these disorders' pathogenicity (Bartzela et al., 2017).

The interdisciplinary team treating patients with CL/P is evaluating the function and the development of the craniofacial structures.

Deep phenotyping and 3D face imaging may assist in mechanistic or early genetic diagnosis (Lewyllie et al., 2018). Identifying genetic variants related to these patients may advance personalized health care (Bartzela et al., 2017).

OFCs and associated malformations may raise suspicion for other related disorders and syndromes. The most common associated malformations are in the skeletal, urogenital, cardiovascular, and central nervous systems (Stoll, 2000; Kouwenberg et al., 2010; Bartzela et al., 2021).

In a sample of 460 patients with OFC, the incidence of patients with cardiovascular anomalies was five times higher than in the general population (Stoll, 1985). Some congenital heart defects are life-threatening; therefore, an early diagnosis of these conditions is vital.

Close monitoring of these patients is essential because some malformations or disorders can be identified later in life. Syndromes such as the 22q11DS (22q11 deletion syndrome) may be suspected and diagnosed as learning disabilities or psychiatric disturbances that are apparent or recognized in later developmental stages (Bassett et al., 2011).

The integrated approach and long-term treatment planning from the multidisciplinary team may assist in the best treatment outcome. Interdisciplinary care is needed until the end of craniofacial growth and, in many conditions, even longer.

Nevertheless, treatment is complicated because of knowledge gaps and dental and craniofacial developmental phenotypic variations.

The etiopathogenesis should be considered, especially in patients with myotonic, nerve palsy, or facial expression disorders. A valuable tool for evaluating the craniofacial development of non-affected individuals and craniofacial anomalies is 3D stereophotogrammetry (Brons et al., 2013).

Affected individuals have a higher morbidity and mortality rate than non-affected (Ngai et al., 2005; Christensen et al., 2004). The main reasons are the associated congenital malformations (61%) and infections (17%) (Kang et al., 2012).

Moreover, dental phenotyping is only possible at 12-14 years of age after the eruption of all permanent teeth (Bartzela et al., 2017). However, phenotyping of the craniofacial features is possible only after growth cessation of the craniofacial complex, at about 18 years of age (Bartzela et al., 2017).

TA is more common in CLP patients than in the general population (Polder et al., 2004). TA might be attributed to developmental factors, such as tissue insufficiency in the cleft area or iatrogenic interventions related to some surgical procedures (Lekkas et al., 2000). The increased prevalence of TA outside the cleft region in the maxilla and the mandible implies a common genetic pathway of the two conditions (Bartzela et al., 2013). Most patients with OFC need complex and long-term orthodontic treatment with an MBA.

The orthodontic treatment focuses on expanding the dental arch and establishing symmetry of the collapsed alveolar segments before the secondary bone grafting. According to the protocol of the Charité – Universitaetsmedizin Berlin, Campus Mitte, the patients received autologous bone harvested from the iliac crest. The secondary autologous bone grafting (SABG) was performed before the lateral or canine eruption, depending on the cleft's location. The grafted cancellous bone is radiographically homogenous with the host bone after approximately three months (Bartzela et al., 2020). The OTM on the cleft side can then start actively through the grafted bone.

The EARR has been evaluated in mature and immature bone (Nakamoto et al., 2002). The radiographic evaluation of the maxillary incisors six months after the orthodontic treatment initiation with an MBA may point out the patients at risk (Smale et al., 2005).

The paralleling technique has been used as a standardized method for evaluating EARR, but the OTM (torque) may bias the results (Chan and Darendeliler, 2004; Årtun et al., 2009; Barros et al., 2017). CBCT is considered more reliable for the diagnosis of EARR (Aras et al., 2018b). Nevertheless, it should be used for EARR only if available (Barros et al., 2017) because of ALARA restrictions.

Preexisting EARRs (Jiang et al., 2010), initial atypical root form (Mirabella and Årtun, 1995b; Årtun et al., 2005), and oral habits (e.g., lip- or tongue posture or dysfunction) (Pandis et al., 2008b) have been considered risk factors for EARR during the orthodontic treatment. Furthermore, diseases (osteoporosis, hyperthyroidism, etc.) or medication (bisphosphonates, estrogen hormone therapy, etc.) that affect bone metabolism are associated with an increased EARR (Verna et al., 2003).

Patients with CLP need a complex and extended orthodontic treatment due to a lack of arch continuity (Bartzela et al., 2020), rotated incisors on the cleft side, and collapsed asymmetric arches. Severe EARR due to orthodontic treatment is relatively rare but more often seen on the cleft side's central incisors.

In the early stages of OTM, corticotomies and corticisions may decrease the susceptibility to EARR (Peron et al., 2017).

Follow-up of patients with CL/P or intercenter studies to evaluate the treatment outcome can be performed with CDs (Compact Discs**)** or e-mail transfer of the photographic representation of the dental casts or digital models (Leenarts et al., 2012).

This method can enable us to organize easier and more efficient intercenter studies or remote clinical audits. The images could be placed on a password-protected website allowing other researchers or accredited raters anywhere in the world to score the treatment outcome. With training packages for new users, this method could increase the accessibility of regional Good Practice Archives.

Early identification of patients' associated medical problems with CL/P, TA, or EARR will lead to accurate diagnosis and long-term treatment intervention from the clinical perspective. The team's goal is to overcome existing clinical challenges that hamper a satisfying functional and aesthetic outcome, improving the patients' and their families' satisfaction and, above all, their quality of life.

3.4 Conclusions and recommendations for further research

The complexity and severity of orofacial clefts (OFCs) require centralization, multidisciplinary teamwork, coordination of care, collective decision-making, and long-term integrated planning.

Early fetal diagnosis and genetic counseling may help specialized postpartum care, improve treatment outcomes, reduce postpartum mortality rate, and emotional preparation for parenthood.

Early identification of a co-existing condition is fundamental for preventing life-threatening complications and modifying craniofacial growth. Learning disabilities, psychiatric disorders, or heart diseases may be diagnosed at a later age (Shprintzen et al., 1978), (Bartzela et al., 2021). Therefore, the clinical phenotypes of these patients should be reexamined in different developmental stages.

The severity of the cleft is closely associated with a high rate of dental anomalies. The clinical phenotype of patients with CL/P and TA patterns might help identify the responsible genetic factors (Bartzela et al., 2010; Bartzela et al., 2013).

Deep phenotyping of dental and craniofacial structures is possible only after the cessation of these structures' growth and development (Bartzela et al., 2017).

Early interventions and decisions concerning the type and timing of orthodontic treatment (Bartzela et al., 2020) and maxillofacial surgery are often challenging and critical, especially in patients with disrupted craniofacial and dental structures development (Bartzela et al., 2017).

Patients with CLP need a complex and long-term orthodontic treatment with MBA. Identifying anatomical aspects of the bone and teeth close to the cleft area may elucidate the predisposing factors associated with EARR (Bartzela et al., 2020). The extrapolation of results derived from experimental animal studies in humans should be considered cautiously (Bartzela et al., 2009). Further clinical trials are still required to understand better the molecular basis of EARR (Spoerri et al., 2018).

The use of the BCLP yardstick was evaluated in three age groups, and three formats of dental models were used. The photographs and digital models are compared with conventional dental casts. They can assess dental arch relationships in patients with cBCLP using BCLP yardsticks at 6, 9, and 12 years of age. The overjet and crossbite assessment in 2D photographs was more difficult than in dental casts or digital models. The last two formats can be manipulated and oriented on different angulations facilitating the treatment outcome assessment. The cleft audit

protocol or inter-center comparisons can be achieved with a CD or e-mail transfer of the dental casts, simplifying this kind of studies.

Patients with OFC have more frequently associated congenital malformations and syndromes. The team members should be alerted and examine these children in different developmental stages to diagnose an associated malformation or syndrome. Due to scientific knowledge gaps, there is a lack of consensus for these patients' treatment management. Personalized treatment care should be based on patients' variations and needs or priorities.

The overall prevalence of individuals with CL/P and their pedigrees with associated malformations and syndromes emphasizes the need for early identification, interdisciplinary interaction, and long-term planning. Phenotypic variability in pedigrees makes the understanding of etiopathogenesis more difficult. These pedigrees did not propose a particular inheritance trait, but it was produced for further molecular analysis.

Genome-wide linkage analysis has already been performed for pedigrees 6 and 10 (SI 2) (Mangold et al., 2009). Furthermore, genetic analysis of the other pedigrees will be performed for the identification of susceptible loci.

Clinical trials and epidemiological data may help to identify the role of genetic variation, clinical phenotypes, bias, and confounding factors. The team's goal is to ascertain functional and aesthetic outcomes and, above all, the quality of life of these patients and their families.

Further research is required for clinical recommendations and treatment consensus.

Assessment of the cost-effectiveness of the treatment of these patients is complicated. Most of the time, the calculated costs are hospital or insurance charges that often do not represent the actual expenses. Therefore, the disciplines involved in the team should provide cost and outcome assessments.

4. Summary

Patients with an orofacial cleft (OFC) need complex treatment and require long-term and collective decisions from the multidisciplinary team. These patients may have an associated congenital malformation or syndrome. Some conditions like learning or psychiatric disorders or heart diseases can be diagnosed later in life. Therefore, the team members should examine the patients in different developmental stages to diagnose early an associated condition. Nevertheless, deep phenotyping of the dental and craniofacial affecting structures is only possible after 14 and 18 years of age, respectively, at the end of these structures' development. Moreover, the genetic basis of OFC is multifactorial and, so far, not well understood. In our study, almost 25% of the examined patients with OFC had a positive family history (Bartzela et al., 2021). Patients with CPO have an associated anomaly or syndrome (66,7%) more often than patients with other cleft types. Skeletal (27,7%) and eye malformations (22,9%) were more common than malformations in other organs or systems. Sex predominance has been registered according to the cleft type. Males were more often observed in CL/P (71,4%) and females (59,7%) in CPO cleft types. Phenotyping CL/P pedigrees make understanding etiopathogenesis even more complicated (Bartzela et al., 2021).

The increased prevalence of TA in these patients inside and outside the cleft region of the maxilla but also in the mandible suggests a common genetic pathway between OFC and TA.

Patients with OFC require long-lasting, multidisciplinary orthodontic treatment with an MBA. Expansion and symmetry of the collapsed dental arches, followed by a secondary bone graft to correct the interrupted arch continuity, are required before the orthodontic tooth movement in the cleft region. The severe form of EARR related to orthodontic treatment is rare, and the most commonly affected teeth are the central incisors of the cleft side (Bartzela et al., 2020).

Severe rotation of the central incisors on the cleft side, pre-existing EARR, atypical root form, specific bone anatomical aspects, oral habits (e.g., lip or tongue posture or dysfunction), and associated medical conditions have been considered as predisposing factors for EARR during the orthodontic treatment (Bartzela et al., 2020).

Intercenter studies for evaluating treatment outcomes of patients with CLP can be carried out with e-mail transfer or CDs of images of the dental casts or digital models.

The images could be protected by a password, allowing the participant raters from their computers to access and score the dental models. This method facilitates intercenter studies or remote clinical audits.

Clinical trials, intercenter studies, and epidemiological data may help evaluate treatment results and identify the role of genetic variation bias and confounding factors on clinical phenotypes. Documentation and meticulous record collection of these patients for the quality assessment of the treatment outcome are mandatory. Early identification of associated syndromes or malformations is of vital importance, especially in life-threatening complications.

The type and timing of orthodontic treatment and maxillofacial surgery are critical and often complicated by disrupting dental and craniofacial structures' development.

The team's goal is to improve function and aesthetic outcomes and ascertain a better quality of life for these patients and their families. Patients' and parents' considerations, satisfaction, and psychosocial aspects need research evaluation. Further research is required for clinical recommendations and treatment consensus.

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6. List of abbreviations

22q11DS	22q11 deletion syndrome
2D or 3D	two dimensional or three dimensional
ADAM syndrome	“amniotic deformity, adhesions, mutilations” syndrome
ALARA	as low as reasonably achievable
BBB- syndrome	the first letters of the last names of the families first diagnosed with this disorder
BCOR-gene	BCL6 corepressor
BCLP or BCL/P	bilateral cleft lip and palate or / bilateral cleft lip and palate and/or palate
cBCLP	complete bilateral cleft lip and palate
CBCT	cone beam computed tomography
CD	compact disc
CLP or CL/P	cleft lip and palate or cleft lip and/or palate
CLO	cleft lip only
CNS	central nervous system
CPO	cleft palate only
CSO	cleft soft palate only
cUCLP	complete unilateral cleft lip and palate
EARR	external apical root resorption
EEC syndrome	ectodermal dysplasia ectrodactyly cleft syndrome
ENT	ear, nose, throat
FGFR1	fibroblast growth factor receptor 1
CHARGE syndrome	“coloboma, heart anomaly, choanal atresia, retardation, genital and ear anomalies” syndrome
HB system	Huddart/Bodenham system
IMR	infant mortality rate
ICDFA	international database on craniofacial anomalies
IO	infant orthopedics
IRF6	interferon regulatory factor 6
MA	meta-analysis
MBA	multibracket appliance
NVSCA	Dutch association for cleft palate and craniofacial anomalies
nsCL/P or sCLP	non-syndromic CLP
OFC or OFCs	Orofacial cleft or orofacial clefts

OFCD	Occulo-facio-cardio-dental syndrome
OTM	Orthodontic tooth movement
OPGs	Orthopantomograms
P63	tumor protein p63, also known as transformation-related protein 63 (*tp63)*
PA	periapical radiographs
PBX1	pre-b-cell leukemia transcription factor 1
PBX2	pre-b-cell leukemia transcription factor 2
PRS	Pierre Robin sequence
PRS-plus	Pierre Robin sequence with multiple congenital anomalies
Radboudumc	Radboud university medical centre
RCT	randomized controlled trial
sCL/P or sCLP	syndromic cleft, lip and/or palate or syndromic cleft lip and palate
SI	supplementary information
sPRS	syndromes associated with Pierre Robin sequence
SR	systematic reviews
TA	tooth agenesis
TAC	tooth agenesis code
TBX22	t-box transcription factor 22
Tcof1	treacle ribosome biogenesis factor 1
TP63	tumor protein p63, typically referred to as p63, also known as transformation-related protein 63
UCLP or UCL/P	unilateral cleft lip and palate or unilateral cleft lip and/or palate
vs.	versus
VWS	van der Woude
WHO	World Health Organization
yrs.	years

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8. Erklärung

Erklärung

§ 4 Abs. 3 (k) der HabOMed der Charité

Hiermit erkläre ich, dass

- weder früher noch gleichzeitig ein Habilitationsverfahren durchgeführt oder angemeldet wurde,
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