

# Recurrent Aphthous Stomatitis in Children: A Practical Guideline for Paediatric Practitioners

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## ABSTRACT

Recurrent aphthous stomatitis (RAS) is the most common chronic oral mucosal lesion affecting up to 25% of the population. The diagnosis is based on well-defined clinical characteristics, but the precise aetiology and pathogenesis remain unclear. The treatment of RAS should be based on the identification and control of possible predisposing factors. A wide range of topical medicaments is available as antiseptics, anti-inflammatory drugs and corticosteroids. The systemic treatment is indicated in patients with continuous and aggressive manifestation, which is extremely rare in children. The present article provides a review of the current concept and knowledge of the aetiology, pathogenesis, and management of RAS in the paediatric population.

## KEYWORDS

recurrent aphthous stomatitis; children; pathogenesis; treatment

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## INTRODUCTION

Aphthae (cancer sores) is one of the most commonly recorded painful lesions in the oral cavity and were first mentioned by Hippocrates (460–370 BC) who utilized the term “aphthai” (1). Recurrent aphthous stomatitis (RAS) is characterized by multiple recurrent small, round, or ovoid ulcers with circumscribed margins, erythematous haloes, and yellow or grey floors. They typically present in children and adolescents (2). Up to 25% of the population can be affected by aphthae. It is a disease with high recurrence rate (50% at 3 months). Aphthae are more common in females (3). Many predisposing factors have been identified. These include especially local trauma, genetic factors, nutritional deficiencies, viral and bacterial infections or immune and endocrine disease (4). All forms of aphthous ulcers have a significant impact on the quality of life and interfere with the child’s well-being.

## PATHOGENESIS

Several theories have described the pathogenesis of RAS. Significant interactions between the immune system, genetics and environmental factors play a significant role. DNA damage secondary to oxidative stress is thought to play a role in RAS (5). Evidence suggests an immunological basis for chronic inflammation. T cell-mediated immunity plays a significant role in RAS development. The imbalance between CD4+ and CD8+ T lymphocytes (decreased ratio) is very frequent observation. In patients with RAS. T cells are responsible for epithelial destruction via generated TNF- $\alpha$ . TNF- $\alpha$  has been found to be significantly increased in the saliva of RAS patients. A recent study explored the significance of single nucleoid polymorphisms in the genes for pro-inflammatory cytokines IL-1 and IL-6. This suggests a genetic component to the immuno-pathogenesis of RAS (6). The results of the recent case-control study strongly indicated that RAS patients have a systemic imbalance in the oxidant-to antioxidant ratio favouring oxidative damage (7). It is currently thought that an

**Tab. 1** Systemic disorders associated with RAS (25).

Behcet’s syndrome
Celiac disease
Cyclic neutropenia
Nutritional deficiencies (iron, folate, zinc, B1, B2, B6, B12)
IgA deficiency
Immunocompromised conditions, including HIV disease
Inflammatory bowel disease
MAGIC syndrome (mouth and genital ulcers with inflamed cartilage)
PFAPA syndrome (periodic fever, aphthous stomatitis, pharyngitis, cervical adenitis)
Reactive arthritis
Sweet’s syndrome
Ulcus vulvae acutum

unknown antigen stimulates keratinocytes, resulting in cytokine secretion and leukocyte chemotaxis. The RAS may also be associated with a specific HLA haplotype such as HLA-A2, A11, B12 and DR2 (8).

## PREDISPOSING AND ENVIRONMENTAL FACTORS

### LOCAL FACTORS

Local trauma during mastication or tooth brushing is regarded as a possible cause of RAS (2, 9). Trauma predisposes to RAS by inducing oedema, early cellular inflammation associated with increased viscosity of the oral submucosal extracellular matrix (10). Some changes in salivary composition, such as pH, and stress-induced salivary cortisol have been correlated with RAS (11).

### DRUGS

Boulinguez et al. reported the association between some drugs as non-steroid anti-inflammatory drugs (NSAID) or b-blockers and RAS (12).

### FOOD HYPERSENSITIVITY

Some foods such as chocolate, coffee, peanuts, cereals, almonds, strawberries, tomatoes, and wheat flour (containing gluten) are considered as predisposing factors (13). Besu et al. published the study reporting the strong association between high levels of serum anti cow’s milk proteins and clinical manifestations of RAS (14).

### NUTRITIONAL DEFICIENCY

Nutritional deficiencies associated with anaemia (iron, serum ferritin) have been reported to be common in RAS paediatric patients (15). Deficiencies of vitamin B1, B2, and/or B6 are also common (16).

### HEREDITARY PREDISPOSITION

At least 40% of RAS patients have a familiar history of RAS (17). Children with RAS positive parents have a 90% chance of developing RAS. When patients have a positive family history, they tend to develop recurrent aphthous ulcers at an early age. They aphthous lesions appear more frequently and demonstrate severe symptoms. Studies of identical twins have also shown the hereditary nature of this disorder (18, 19).

### THE SYSTEMIC DISORDERS

Several systemic disorders have been reported to be associated with RAS. The clinical and morphological findings are not distinguishable from those found in healthy individuals. The systemic disorders that are associated with lesions clinically similar to RAS are shown in Table 1. The celiac disease represents one of the frequent associations in children (20). Mucosal aphthosis is a feature of a systemic syndrome that includes recurrent fever with unknown source of infection (21). Such syndromes are referred to

**Tab. 2** Clinical features of minor, major and herpetiform recurrent aphthous stomatitis (RAS) (36).

	Minor RAS	Major RAS	Herpetiform RAS
Gender predilection	Equal	Equal	Girls
Morphology	Round or oval lesions, covered by grey-white pseudomembranes, erythematous halo	Round or oval lesions, covered by grey-white pseudomembranes, erythematous halo	Small, deep ulcers. Irregular contour
Distribution	Lips, cheeks, tongue, mouth floor	Lips, soft palate, pharynx	Lips, cheeks, tongue, mouth floor, gingiva
Number of ulcers	1-5	1-10	10-100
Size of ulcers	<10 mm	<10 mm	2-3 mm
Prognosis	Lesions resolve in 4-14 days, no scarring	Lesions persist >6 weeks, high risk of scarring	Lesions resolve in <30 days, scarring uncommon

as auto-inflammatory diseases as PFAPA (periodic fever, aphthous stomatitis, pharyngitis, cervical adenitis) (22). The RAS can also be part of various neutrophilic dermatoses (23). The stress remains one of the significant factors affecting the immune system and is believed to predispose patients to RAS (24).

**CLINICAL FEATURES**

There are three clinical representations of RAS: minor (<70% of cases), major (10%), and herpetiform ulceration (10%) (Table 2). Recurrent aphthous stomatitis comprises recurrent bouts of one or more rounded, shallow, painful ulcers at intervals of a few months to a few days. Patients may have prodromal symptoms of tingling or burning before the appearance of the lesions. During this initial period, a localized area of erythema develops. Within hours, a small white papule forms, ulcerates, and gradually enlarges over the next 48-72 hours (26).

**MINOR RAS**

Minor RAS (also known as Mikulicz’s aphthae) is the most prevalent form and typically occurs in children who are 5 to 18 years old (27). They affect only non-keratinized parts

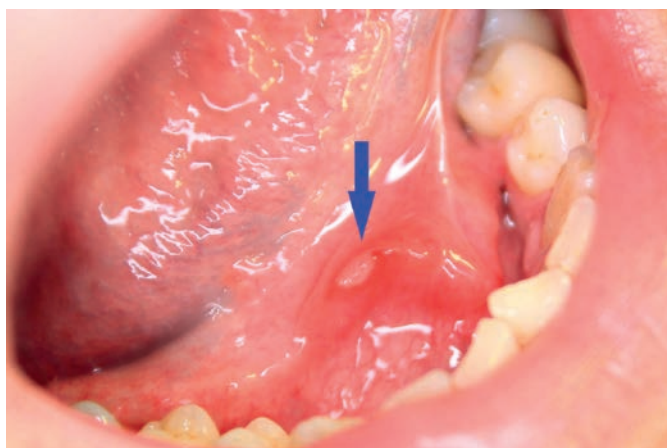
of mucosa. Superficial, round ulcerations are <10 mm, accompanied by a grey pseudomembrane and erythematous halo (Figure 1). They usually occur on non-keratinized mucosa of the labial and buccal mucosa, the floor of the mouth and ventral or lateral surface of the tongue. The ulcers heal within 10-14 days without scarring.

**MAJOR RAS**

Major RAS (or Sutton’s disease or periadenitis mucosa necrotica recurrens) is less common; they are larger than 10 mm in diameter, more profound, often scared (Figure 2). These lesions have a predilection for lips, soft palate and tonsils. Lesions may appear on any mucosal surface. These lesions take up to 6 weeks to heal. The onset is usually after puberty and recurrence can last for decades.

**HERPETIFORM ULCERATION**

Herpetiform ulcerations (or Cooke’s) constitute only 5-10% and are very rare in children (28). These lesions are small and multiple, typically affected lateral margins and ventral surface of the tongue and floor of the mouth. Individual ulcers are grey with an irregular contour. A single crop of ulcers may last for 7-14 days, making eating and speaking difficult (26).



**Fig. 1** Aphtha minor.



**Fig. 2** Aphtha major.

## DIAGNOSIS

No specific diagnostic test exists to diagnose RAS. The correct diagnosis of RAS is dependent on a detailed clinical history and examination of the ulcers. Usually, it does not cause difficulties because of the clinical appearance and recurrence of the lesions. It is necessary to point out the possible problems in the differential diagnosis when aphthous stomatitis is considered as changes typical for herpetic gingivostomatitis with systemic prodromal symptoms that are absent in RAS. Histological examination is characteristic but not specific for RAS. Central ulceration covered by fibro purulent membrane is a frequent finding in early stages (29). Mixed inflammatory infiltrate is present in adjacent connective tissues. The histopathological examination is sometimes necessary to differentiate aphthous ulcers from other mucocutaneous diseases that have ulcerative manifestations such as neoplastic lesions. To rule out potential viral causes such as varicella zoster virus infections, herpangina, hand-foot-and-mouth disease or Coxsackie virus-related oral ulcers it is sometimes necessary to do microbiological examination (either direct or indirect diagnostics). Underlying systemic conditions should be identified (celiac disease, IBD, hematologic disorders, nutritional deficiencies) (30).

## TREATMENT

The current concept of the management of RAS is aimed at supportive care. It is necessary to point out that once the development of lesion starts, it is not possible to stop the pathogenetic process. No pharmacological treatment has been curative, although several modalities have been effective in decreasing pain and erythema and increasing the rate of re-epithelialization of the lesions. The comfort of the treatment procedures (application form, frequency, and discomfort in the oral cavity) should be taken into consideration, particularly in paediatric patients. The positive fact is that children suffer most from minor lesions, but the treatment modalities are limited by the age and cooperation of the child. It is reasonable to begin treatment with topical medication. Topical treatment is aimed at prevention of superinfection, protection of existing ulcers, analgesia, decreasing inflammation, and treating active ulcers. Systemic therapy is exceptional in children. The systemic treatment is considered only in children with immunity defects (26).

### TOPICAL AGENTS

Local anaesthetics (lidocaine, benzocaine, polidocanol) have a benefit in pain relieve. It is particularly important in children when painful lesions may lead to eating difficulties and dehydration. Possible application forms are solutions, gels, and adhesive pastes. A notable fact is a cooperation of the child; therefore, adhesive pastes are preferred in small children.

Antiseptics (chlorhexidine gluconate, benzydamine hydrochloride, triclosan, cetylpyrimidiumchlorid) prevent the secondary infection and may relieve symptoms. The different application forms are available.

Based on the immunologic nature of RAS, topical steroids may often control RAS. Topical triamcinolone or stronger steroids such as betamethasone may be used. Steroids act on the lymphocytes and alter the response of effector cells to precipitants of immunopathogenesis (31).

### SYSTEMIC MEDICATIONS

Systemic treatment is indicated for severe and recurring ulcerations where topical management is not sufficient. Options for systemic treatment include the use of immunomodulatory drugs such as corticosteroids, dapsons, colchicine, tetracycline, thalidomide or biologic agents (such as TNF- $\alpha$  inhibitors). The use of these compounds is significantly limited in children, whereas the use of most of them except steroids is usually contraindicated. Treatment with systemic steroids provides an only transient response of RAS (32). Long term steroid use in RAS is not indicated.

Treatment with vitamin B12 has been suggested. Duration of ulcers and pain has been reduced in the study by Volkov showing a benefit of vitamin B12 administration (33).

One of the possible drugs is pentoxifylline inhibiting TNF- $\alpha$  production and other pro-inflammatory cytokines (34). This drug is, however, not indicated in children up to 18 years of age. Most of other medications for the systemic application have a significant immunosuppressive effect, and their indication is hardly justifiable in children (35).

### OTHER THERAPEUTIC AND PROPHYLACTIC MEASURES

The parents and the child should be informed to minimize the local traumatization of the oral mucosa, modify the diet, eliminate possible allergic agents, and reduce the emergence of stressful situations. Proper oral hygiene in older children is essential.

## CONCLUSION

RAS remains a common oral mucosal disorder in the paediatric population. Its aetiology remains unclear. No specific trigger has ever been demonstrated. There is no safe therapy to ensure no recurrence of RAS. In severe cases, the complex paediatric examination of the child is recommended to eliminate the similarly looking oral manifestation of systemic diseases as anaemias, idiopathic gastrointestinal disturbances (celiac disease) and hypersensitivity to various allergens.

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## CONFLICTS OF INTEREST

None declared.

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# Corneal Graft Success Rates in HSV Keratitis: A Systematic Review

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## ABSTRACT

Herpes Simplex Virus (HSV) has worldwide prevalence. The primary objective of this systematic review was to compare penetrating keratoplasty (PK) and deep anterior lamellar keratoplasty (DALK) regarding the efficacy and complications of the treatment of corneal scarring caused by herpes simplex keratitis. Out of the 469 articles identified during the combined search of the literature based on the PubMed and Cochrane libraries, 10 retrospective and 2 prospective studies published from January 2010 to December 2019 were included. The study outcomes indicated that both surgical approaches resulted in a comparable improvement of visual acuity (VA). However, DALK demonstrated fewer complications in the majority of studies. Higher graft survival rates were associated with higher acyclovir (ACV) doses (above 800 mg/day), topical steroid and antibiotic drops. In conclusion, in terms of postoperative VA, both PK and DALK demonstrate comparable efficacy. However, DALK, which is applied in less severe HSK cases, is associated with fewer complications and better graft survival rates. High dosages of ACV, topical steroids and antibiotics contribute significantly to improved postoperative outcomes.

## KEYWORDS

herpes simplex virus; herpes simplex keratitis; penetrating keratoplasty; deep anterior lamellar keratoplasty; systematic review

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## INTRODUCTION

Herpes Simplex Virus (HSV) which belongs to the herpesvirus family is usually asymptomatic, however, it may affect a great variety of organs (1-2). It is estimated that in the U.S. alone 500,000 people suffer from ocular HSV and every year about 50,000 new cases of ocular HSV are diagnosed (3). Following the entry into the host, HSV replicates within the end organ. Following replication, the virus gains the ability to travel up the axon of the corresponding nerve and colonize the corresponding ganglion, where it lies in a latent state (4). For infections involving the face, the trigeminal nerve, which supplies the sensory innervation of the face, and the trigeminal ganglion are involved. In some patients, a number of stimuli, either physical, such as corneal trauma (eye injury, surgery, excimer laser) or other factors such as psychological stress, fever, systemic infection, immunodeficiency, sunlight exposure and menstruation (4-6), trigger reactivation of HSV. As a result, the virus replicates and travels down the axon of the sensory nerve to its target tissue, causing recurrent infection and stimulating an inflammatory response. The spectrum of ocular disease caused by HSV is wide and depends on the target tissue that is infected. Both the anterior and posterior segments of the eye can be involved; among them, herpetic blepharitis, conjunctivitis, keratitis, as well as herpetic uveitis (iridocyclitis or trabeculitis) are some possible manifestations. In the most severe and rare cases, necrotizing herpetic retinopathy may occur with devastating outcomes to the visual capacity.

Regarding corneal disease, Herpes Simplex Keratitis (HSK) is the leading cause of corneal infectious blindness in developed countries (7). HSV has the ability to infect all the layers of the cornea and lead to infectious epithelial keratitis, neurotrophic keratopathy, necrotizing stromal keratitis, immune stromal keratitis and/or endothelitis. The most typical lesion of HSK is the dendritic ulcer and the geographic ulcer in severe cases; both can be stained positively with fluorescein. Other examinations such as polymerase chain reaction (PCR), tear collection and immunofluorescence antibody assay (IFA) have also been used in order to identify the virus (4). Corneal epithelium involvement may occur in up to two thirds of the cases with herpetic ocular disease (8). However, the relapsing and recurring disease of stroma and endothelium is responsible for most of the cases of corneal scarring and neovascularization. Disciform keratitis is related to about 2% of initial ocular HSV presentation. Nevertheless, it is responsible for 20-48% of disease recurrences (8-9). Herpetic keratitis can result in reduction of visual acuity (VA) to <6/12 in 10-25% and corneal scarring in 18-28% (9). Patients suffering from HSK usually have a red painful eye accompanied by other symptoms such as discharge, irritation, itching, watery eyes and photophobia.

Acyclovir (ACV), either in topical, oral or intravenous form, remains until today the mainstay of treatment against all herpes ocular disease's types. ACV can be used in combination with corticosteroids or other antiviral drugs like ganciclovir (GCV) or valaciclovir (VCV). However, oral or topical drugs do not eradicate the virus but only lower the risk of recurrence of ocular disease. In case

of HSV-induced corneal opacities, surgical debridement may be indicated (4).

Deep anterior lamellar keratoplasty (DALK) is a surgical procedure in which the pathological stroma is excised down to Descemet membrane (DM), leaving the original corneal endothelium intact. As a result, DALK can be used for the treatment of corneal scarring when the endothelium and DM have not been affected (10-12). On the other hand, penetrating keratoplasty (PK) is another surgical technique that could be used in HSK-induced corneal scarring, especially in those cases that is complicated with endothelial insufficiency. However, both DALK and PK suffer from a series of adverse effects (13-15); among them, endothelial rejection, cell loss or failure, damage to the iris and/or crystalline lens, microbial endophthalmitis and expulsive choroidal hemorrhage are part of the spectrum of the complications of PK. On the other hand, ruptures or microperforation of DM, double anterior chamber and recurrence of stromal cornea dystrophy in the residual bed are unique complications of DALK. Moreover, epithelial and stromal immune graft rejection, and graft failure can occur with either procedure and are commonly easily managed with topical corticosteroid drops. Corticosteroid-associated high intraocular pressure (IOP), cataract, decreased wound healing, and compromised local immunity are some additional adverse effects of both procedures, however, with DALK having fewer and less severe adverse effects in comparison with PK (16). Thus, apart from PK and DALK, several approaches have been used for the management of HSV keratitis including therapeutic contact lenses, collagenase inhibitors, tarsorrhaphy, conjunctival flap, and cyanoacrylate gluing (17-18).

Within this context, the primary objective of this systematic review was to compare PK and DALK regarding the efficacy and complications of the treatment of corneal scarring caused by HSK.

## MATERIAL AND METHODS

### STUDY DESIGN AND INCLUSION CRITERIA

This systematic review followed the Preferred Reporting Items for Systematic Reviews (PRISMA) statements checklist (19). The inclusion and exclusion criteria were defined before the initiation of the research. Only original articles and case series with 5 or more subjects were included whose main or secondary goal was to demonstrate outcomes regarding DALK or PK or both interventions in populations suffering from corneal scarring as a result of HSK. Commentaries, conference abstracts, editorials, letters to the editor, case series with less than 5 patients were not considered.

The selection criteria were defined by applying the PICO (Problem/Population, Intervention, Comparison, and Outcome) framework. Participants included immunocompetent adult patients (above 18 years old) with corneal scarring as a result of HSK. Intervention consisted of PK or DALK or both and the following postoperative drug administration. Some of the included studies compared PK and DALK, but articles in which intervention were PK or DALK without any comparison were also considered.

Tab. 1 Quality assessment.

Reference	Year of publication	Selection Bias	Study Design	Confounders	Blinding	Data Collection Methods	Withdrawals and Drop Outs	Global Rating
Altay et al. (30)	2017	Moderate	Moderate	Strong	Weak	Strong	NA	Moderate
Li J. et al. (32)	2011	Moderate	Moderate	Strong	Weak	Strong	Moderate	Moderate
Li J. et al. (31)	2014	Moderate	Moderate	Strong	Weak	Strong	NA	Moderate
Li S. et al. (22)	2019	Moderate	Moderate	Strong	Weak	Strong	Strong	Moderate
Liu et al. (26)	2016	Moderate	Weak	Strong	Weak	Strong	NA	Weak
Lyall et al. (27)	2012	Moderate	Weak	Weak	Weak	Strong	NA	Weak
Ren et al. (28)	2016	Moderate	Weak	Strong	Weak	Strong	NA	Weak
Sarnicola et al. (21)	2010	Moderate	Weak	Strong	Weak	Strong	NA	Weak
Shimizu et al. (23)	2017	Moderate	Weak	Strong	Weak	Strong	NA	Weak
Wang et al. (29)	2012	Moderate	Weak	Strong	Weak	Strong	NA	Weak
Wu et al. (24)	2012	Moderate	Moderate	Strong	Weak	Strong	NA	Moderate
Zheng et al. (25)	2009	Moderate	Weak	Strong	Weak	Strong	NA	Weak

NA: Not applicable

DALK was primarily indicated when the endothelial layer and the DM of the cornea remained healthy with no sign of stromal edema, while PK was indicated when all corneal layers (epithelium, stroma and endothelium) were affected or, in specific, when corneal endothelial cell count was  $<700$  cells/mm<sup>2</sup> or was undetectable. Primary outcomes included rate of rejections and VA. Secondary outcomes included rate of recurrence, graft failure, microperforation, double minor anterior chamber, graft melting and any other complications that were reported.

#### LITERATURE SEARCH STRATEGY

A literature search was performed based on the PubMed and Cochrane libraries using the following search terms: (HSK OR herpes simplex keratitis OR herpes OR herpes simplex virus OR HSV) AND (corneal scar OR PK OR penetrating keratoplasty OR DALK OR deep anterior lamellar keratoplasty). Moreover, the reference lists of the eligible studies and relevant review articles were cross-checked to identify additional pertinent studies. We retrieved articles published in English, French and German from January 2010 to February 2020 that met the selection criteria.

#### STUDY SELECTION AND QUALITY ASSESSMENT

The records found were checked for duplicates. Then, two independent reviewers who were blinded to each other decisions screened the articles first by title and abstract and after that full-text screening was conducted. Any conflict was dissolved by a third reviewer. Risk of bias of the eligible articles was conducted with “Quality Assessment Tool for Quantitative Studies” by Effective Public Health Practices (20). Again, the same two individual reviewers assessed the articles, blinded to each other’s decisions and any conflict was resolved by a third reviewer. The results are demonstrated in Table 1.

#### DATA EXTRACTION

Data extraction was also carried out by (K.S.) and (E.C.), blinded to each other’s decisions and (G.L.) resolved any conflict. The following information were noted: author, year of publication, study location, study design, total patients enrolled in the study, total patients who completed the study, patient demographic characteristics, treatment groups, dose and schedule of interventions, duration of follow-up, primary outcomes (rejection rate for the first rejection episode, VA) and complications.

#### RESULTS

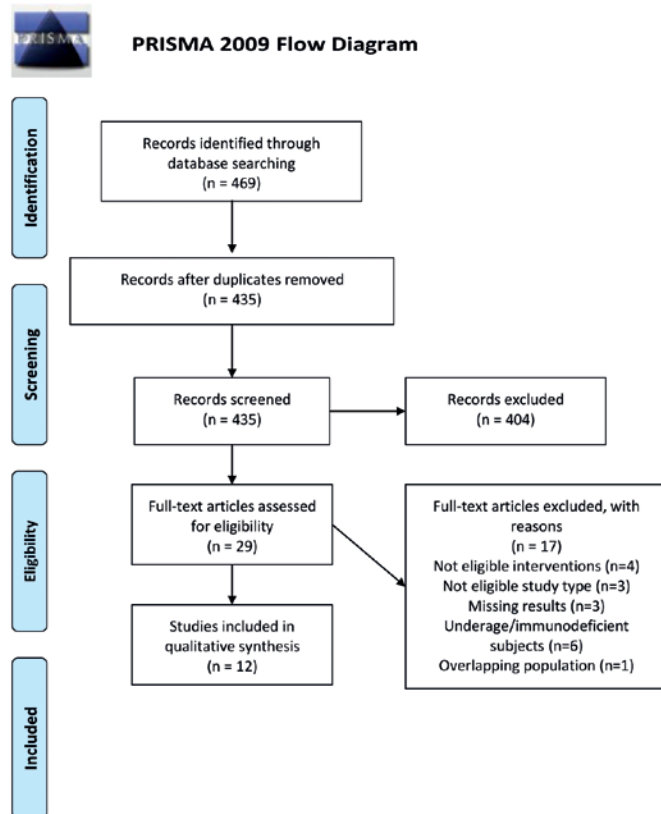
##### LITERATURE SEARCH AND SELECTION

Overall, the combined search identified 469 articles. After the removal of duplicates, 435 studies remained. Our criteria were matched in 29 records and they were assessed in full-text form for eligibility. No additional study was identified through cross-check of reference lists. Out of twenty-nine articles, 17 were excluded due to the following reasons; 6 because of underage or immunodeficient subjects, 3 studies because they were not an acceptable article type, 3 because of missing results, 1 due to overlapping population and 4 records because they did not apply the eligible interventions (DALK or PK). A PRISMA flow chart is demonstrated in Figure 1. Finally, the 12 remaining articles were assessed for quality as it was described before and a summary of the results is demonstrated in Table 1.

##### STUDY CHARACTERISTICS

The 12 selected studies were published from January 2010 (21) to December 2019 (22). The present review included 10 retrospective (21, 23–31) and 2 prospective studies (22, 32). The record’s subjects varied from 13 eyes of 13 patients





**Fig. 1** PRISMA 2009 flow diagram.

Source: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi: 10.1371/journal.pmed1000097.

(25) to 121 eyes of 121 patients (24). DALK, using Anwar's big bubble technique (33), was described as the only intervention in 4 records (21, 27–29) (Table 2). Other research teams performed: DALK using acellular corneal tissue (ACT) (32), DALK using pre-cut anterior lamellar cap (ALC) (31), acellular porcine corneal stroma (APCS) DALK (22), or DALK using glycerol-cryopreserved corneal tissues (GCCTs) (26) (Table 3). PK as the only intervention was carried out by Altay et al. (30). Shimizu et al. (23), in their comparative study, divided the participants in two groups, one in which PK was performed and another one in which DALK was applied. Wu et al. (24) described case series comparing full-bed deep lamellar keratoplasty (full-bed DLK) and PK (Table 4). In the case series of Zheng et al. (25), PK and DALK were performed but with no comparison between interventions. The extracted data of these 12 articles are shown in Tables 2–4. The postoperative prescriptions are described in “dose and schedule of intervention” section of these tables.

#### PRIMARY OUTCOMES

Regarding DALK procedure (Table 2), a significant improvement in vision after the operations was described (21, 27–29). The rejection was lower than 5% in the studies of Ren et al. (28) and Wang et al. (29) in a 50.4-month and 31-month follow-up period, respectively, while no rejection was detected in a 31-month follow-up period according to

Sarnicola et al. (21) records. However, in the study of Lyall et al. (27), the rate of rejection was 50% during a follow-up period of 56-months. Rejection rate lower than 12% was noted in DALK using ACT (32) and glycerol-cryopreserved corneal tissues (GCCTs) (26). On the other hand, the rejection was 0% in DALK using pre-cut ALC (31) and APCS (22) (Table 3). Regarding the postoperative VA, a significant improvement was observed postoperatively after DALK using pre-cut ALC (31) and DALK using GCCTs (26). The VA outcomes in DALK using ACT (32) and in APCS DALK (22) presented in Table 3 were mixed with other types of keratitis. In a case series of Zheng et al. (25), with mixed results of DALK and PK using APCS, VA was improved in 69.2% of the population and no rejection occurred. In the study of Shimizu et al. (23), PK and DALK procedures achieved similar improvement in VA but no data about the complications were given. PK showed graft rejection lower than 10% in the study performed by Altay et al. (30) and 93% of the patients achieved BCVA better than 1.2 logMAR. Last but not least, in the comparative study of Wu et al. (24), PK showed a significantly higher number of graft rejections when compared with full-bed DLK (41.3% and 0%, respectively,  $p < 0.05$ ). Moreover, the VA was improved in 66.1% of the eyes that received full-bed DLK and 50.9% in the PK group.

#### SECONDARY OUTCOMES

In DALK procedure, HSK recurrence was observed from 0% (21) to 33.3% (27) of the study population. Microperforation in DALK occurred in 3.8% (21) to 16.9% (28) of the subjects. In the study of Lyall et al. (27), increased IOP, bacterial keratitis and graft failure was found in 27.7% of the patients. Several complications including double anterior chamber, corneal endothelial decompensation and posterior stromal folds were described in 10.1%, 3.4% and 46.1% of the population, respectively, in Ren et al. (28) records. No recurrence was noted in patients who had DALK procedure using ACT (32). In DALK using pre-cut ALC, microperforation was 7.4% in the FTS group and 4.7% in the ALC group, double anterior chamber was 14.8% in the FTS group and 4.7% in the ALC group, DM folds were 7.4% in the FTS group and 14.3% in the ALC group and recurrence was observed in 11.1% of the population in the FTS group and 14.3% in the ALC group (31). In APCS DALK procedure, graft failure was described in 57.1% of the patients, recurrence in 14.2% and graft melting in 42.6% (22). In DALK using GCCTs, different complications were observed; among them, microperforation (14.8%), double anterior chamber (7.4%) and recurrence (7.4%) (26). Regarding PK procedure, in the study of Altay et al. (30), among 55 patients, recurrence of HSK was noted in 28.5%. In group 1, which included patients with quiescent herpetic corneal scar, graft failure was observed in 19%, and graft rejection in 9.52%, while in group 2, which included patients with a corneal descemetocoele or perforation, graft failure was noted in 30.8% and graft rejection in 23.07% (30). According to Zheng et al. (25), who performed PK and DALK with APCS in different patients, the overall recurrence rate was 23% and inflammation occurred in 7.6% of the subjects. Wu et al. (24), who compared full-bed DLK with PK, noted

Tab. 2 DALK with corneal allograft.

Procedure type	DALK with corneal allograft (graft type not exactly defined)			
Author	Lyall et al. (27)	Ren et al. (28)	Sarnicola et al. (21)	Wang et al. (29)
Year of publication	2012	2016	2010	2012
Study location	Glasgow, UK	Zhejiang, China	Grosseto, Italy	Wuhan, China
Study design	Retrospective, descriptive, Case series	Retrospective, Case series	Case series	Retrospective, Case series
Total patients enrolled in the study	18	89	52	42 pts (43 eyes)
Total patients who completed the study	18	89	52	42 pts (43 eyes)
Patient demographic characteristics	Mean age: 57 38.8% male	Mean age 47.1 60.7% male	Mean age 46.5 61.5% male	NA
Treatment groups	1 group	3 groups Folds-off Group (n = 27) Folds-on Group (n = 14) No-folds Group (n = 48)	1 group	1 group
Dose and schedule of interventions	oral ACV 400 mg × 2/d for 12 m topical chloramphenicol 0.5% for 1 m dexamethasone 0.1% eye drops × 4/d for 1 m, tapering for 6–9 m	oral ACV 400 mg × 5/d for 1m, tapering to 400 mg × 2/d for 12 m tobramycin 0.3% / dexamethasone 0.1% eye drops tapering for 3 m	oral ACV 800 mg × 3/d for 2 m, tapering to 800 mg × 1/d for long term topical antibiotics and corticosteroid × 4/d for 1 m dexamethasone drops × 3/d for the next 1 m tapering to drops × 2/d for long term	Preop.: i.v. ACV 250 mg × 3/d for 3 d 0.15% GCV ointment × 1/d for 3 d 0.1% ACV eye drops × 4/d for 3 d tobramycin sulfate eye drops × 4/d for 3 d Postop.: 0.1% ACV eye drops × 4/d for 6 m oral ACV 400 mg × 3/d for 3 m tobramycin 0.3% / dexamethasone 0.1% eye drops × 4/d for 3 w and replaced with 0.02% fluorometholone eye drops × 3/d daily for 5 m then 0.15% GCV and tobramycin eye ointments × 1/d
Duration of follow-up	56 m	50.4 m	31 m	29.1 m
Primary outcomes	Preop. BCVA: logMAR 1.51 Postop. BCVA: logMAR 0.82	Mean preop. BCVA logMAR 1.63 Folds-off Group: logMAR 0.42 Folds-on Group: logMAR 0.48 No-folds Group: logMAR 0.44	Preop. UVA 20/70 Postop. UVA 20/40	Postop. BSCVA – 20/100 to 20/40: 86%
Complications	Recurrence: 33.3% Rejection: 50% IOP: 27.7% Bacterial keratitis: 27.7% Graft failure: 27.7%	Recurrence: 9% (8 pts) Rejection: 4.5% (4 eyes) Microperforation: 16.9% (15 eyes) Double minor anterior chamber: 10.1% (9 eyes) Corneal endothelial decompensation: 3.4% (3 eyes) Posterior stromal folds: 46.1% (41 eyes)	Recurrence: 0% Rejection: 0% Microperforations (ruptures of DM): 3.8% Endothelial cell loss (6–12 m): 205.32 cells/mm <sup>2</sup>	Recurrence: 14% (7 cases) Recurrence: 9.3% first year Recurrence: 16.3% first 2 years Rejection: 2.3% Non-physiologic corneal graft endothelial cell loss or dysfunction was not observed

ACV: acyclovir; BCVA: best corrected visual acuity; BSCVA: Best spectacle-corrected visual acuity; DALK: deep anterior lamellar keratoplasty; DM: descemet membrane; folds-off group: the central stromal folds were peeled off with resultant bare descemet membrane; folds-on group: the folds were not removed because of the occurrence or high risk of descemet membrane perforation; GCV: ganciclovir, IOP: intraocular pressure; m: months; NA: Not applicable; No-folds group: stromal folds were not observed intraoperatively; postop: postoperative; preop: preoperative; pts: patients; UVA: uncorrected visual acuity

different complications such as recurrence, graft failure, microperforation, and increased IOP. Specifically, recurrence was observed in 10.3% and 20.6% with full-bed DLK and PK, respectively, graft failure in 1.7% and 22.22%, microperforation in 13.8% and 0%, and high IOP in 6.9% and

38.1% in full-bed DLK and PK group, respectively. Moreover, secondary glaucoma, cataract and wound dehiscence was observed in 3.4% of the subjects in the full-bed DLK group, in contrast to 4.8%, 32.6% and 7.9% in the PK group.

**Tab. 3** DALK with different graft types.

Procedure type	DALK using ACT	DALK using precut ALC		DALK with APCS	DALK using GCCTs	
Author	Li J. et al. (32)	Li J. et al. (31)		Li S. et al. (22)	Liu et al. (26)	
Year of publication	2011	2014		2019	2016	
Study location	Zhejiang, China	Zhejiang, China		Guangzhou, China	Shanghai, China	
Study design	Prospective, randomized, comparative study	Retrospective, comparative, cohort		Prospective, cohort	Retrospective, Case series	
Total patients enrolled in the study	68	48		39	27	
Total patients who completed the study	68	48		39	27	
Patient demographic characteristics	Mean age 48.2 53/68 male	Mean age: 42.2 FTS: male:63% ALC: male: 62%		Mean age 45.5 25/39 male	Mean age 40.8 66.67% male	
Treatment groups	2 groups Group 1: GCCT Group 2: FCT HSK: 30 pts (GCCT: 15, FCT: 15) Bacterial keratitis: 15 pts (GCCT: 8, FCT: 7) Fungal keratitis: 14 pts (GCCT: 7, FCT: 7) Ocular burns: 9 pts (GCCT: 4, FCT: 5)	2 groups FTS group (DALK with FTS): 27 pts ALC group (DALK with precut ALC): 21 pts		Fungal keratitis 22 (56.4%) HSK 7 (17.9%) Bacterial keratitis 5 (12.8%) Acanthamoeba keratitis 4 (10.3%)	one group (HSK)	
Dose and schedule of interventions	Topical antibiotics and steroids tapering dose oral ACV tapering dose	Tobramycin eye-drops tapering for 3 m topical steroids (dexamethasone) oral ACV 200 mg × 5/d for 3 m, tapering to 400 mg × 2/d for 12 m		Oral ACV 400 mg × 2/d for 12 m Topical antiviral medications (GCV and interferon) for at least 3 m	Preop.: oral ACV 400 mg × 5/d 0.15% GCV ophthalmic gel × 4/d for 3 w tobramycin sulfate eye drops × 4/d for 3 w 0.02% fluorometholone eye drops × 2 for 3 w Postop.: oral ACV 400 mg × 5/d in the first months, tapering to 400 mg × 2/d for 12–18 m 0.15% GCV ophthalmic gel × 4/d for 6–12 m tobramycin dexamethasone eye drops × 4/d for 1 m, substituted for 0.02% fluorometholone eye drops × 3/d from then on	
Duration of follow-up	24 m	36 m		12 m	24.4 m	
Primary outcomes	<i>(Mixed results for all corneal diseases)</i> Preop. BCVA < 20/200: – GCCT: 78.8% (26/33) – FCT: 74.2% (23/31) Postop. BCVA (24 m) – improved in all cases ≥ 20/40: – GCCT: 57.6% (19/33) – FCT: 54.8% (17/31)	Preop. BSCVA: logMAR 1.21 Postop. BSCVA: – FTS: logMAR 0.26 – ALC: logMAR 0.28		<i>(Mixed results for all corneal diseases)</i> Preop. BCVA: ≥ 20/40: 10.3% Postop. BCVA: ≥ 20/40: 51.2% at 12 m	Preop.: BSCVA HM / 10 cm to 0.15 decimals Postop.: BSCVA 0.41	
Complications	Recurrence: 0% Stromal rejections: – FCT group: 3 pts – GCCT group: 0 pts	Recurrence: Rejection: Microperforations: Double anterior chamber: DM folds:	FTS 11.1% 0.0% 7.4% 14.8% 7.4%	ALC 14.3% 0.0% 4.7% 4.7% 14.3%	Recurrence: 14.2% Rejection: 0% Graft failure: 57.1% Graft melting: 42.6%	Recurrence: 7.4% Rejection: 11.1% Microperforation: 14.8% Double anterior chamber: 7.4%

ACV: acyclovir; ACT: Acellular Corneal Tissue; ALC: anterior lamellar cap; APCS: acellular porcine corneal stroma; BCVA: best corrected visual acuity; BSCVA: Best spectacle-corrected visual acuity; DALK: deep anterior lamellar keratoplasty; DM: descemet membrane FCT: fresh corneal tissue; FTS: full-thickness stroma; GCCT: glycerol-cryopreserved corneal tissue; GCV: ganciclovir; HM: hand movement; HSK: herpes simplex keratitis; m: months; postop: postoperative; preop: preoperative; pts: patients; w: weeks

Tab. 4 PK, PK vs DALK or full-bed DLK.

Procedure type	PK	PK and DALK	Full-bed DLK and PK		PK and DALK with APCS
Author	Altay et al. (30)	Shimizu et al. (23)	Wu et al. (24)		Zheng et al. (25)
Year of publication	2017	2017	2012		2019
Study location	Ankara, Turkey	Chiba, Japan			Hangzhou, China
Study design	Retrospective, Cohort	Retrospective, comparative, Case series	Retrospective, comparative, interventional case series		Retrospective, Case series
Total patients enrolled in the study	55	52	121		13
Total patients who completed the study	55	52	121		13
Patient demographic characteristics	Group 1: mean age 44.51 78.6% male Group 2: mean age 41.76 84.6% male	Mean age 63.3 35.3% male	Full-bed DLK: mean age 42.4, male 37/58 PK mean age 48.8, male 44/63		Mean age 61.9 69.2% male
Treatment groups	Group 1: (42 pts) quiescent herpetic corneal scar Group 2: (13 pts) corneal descemetocele or perforation	Control group: 18 pts PK: 17 pts DALK: 17 pts	Full-bed DLK: 58 eyes PK: 63 eyes		one group (PK only in 5 pts with perforation, DALK in 8 pts with corneal scar)
Dose and schedule of interventions	Topical 0.3% ofloxacin 0.1% dexamethasone sodium phosphate eye drops × 6/d Topical steroids for 12 m, tapering doses Oral ACV 400 mg × 5/d for 3 m, tapering to 400 mg × 2/d for 12 m	NA	Full-bed DLK: 0.3% ofloxacin or 0.5% levofloxacin and 0.1% fluorometholone eyedrops × 4/d topical steroids for 12 m, tapering dose 200 mg topical ACV × 5/d for 3 m, then 400 mg × 2/d for 12–18 m PK: 0.1% dexamethasone sodium phosphate combined with 0.3% tobramycin eyedrops × 4/d topical steroids for 12 m, tapering dose 200mg topical ACV × 5 d for 3 m, then 400 mg × 2/d for 12–18 m		oral ACV 0.4 g × 5/d for 1 m, tapering to × 2/d for 12 m antiviral eye ointment × 4/d for 1 m, tapering to × 2/d for 3 m Corticosteroid eye drops × 4/d for 3 m, tapering dose for long term Artificial eye drops × 4/d for 12 m
Duration of follow-up	Group 1: 19.8 m Group 2: 26.15 m	12 m	46 m		15.1 m
Primary outcomes	Preop. BCVA: HM to logMAR 0.7 Postop. BCVA: – Group 1: 93% BCVA ≥ logMAR 1.2 – Group 2: 100% BCVA ≥ logMAR 1.2	Control: logMAR –0.03 Preop.: – DALK: logMAR 1.16 – PK: logMAR 1.65 Postop.: – PK: logMAR 0.48 – DALK: logMAR 0.44	Preop. BCVA: – Full bed DLK: 0.05 decimals – PK: CF Postop. BCVA: – Full-bed DLK: improvement in 66.1% eyes – PK: improvement in 50.9% eyes		Prep. VA: LP to 0.02 decimals Postop.: – improvement : 69.2% – no improvement: 30.8%
Complications	Recurrence of HSK: 28.57% Group 1: – Graft rejection: 9.52% – Graft failure: 19% Group 2: – Graft rejection: 23.07% – Graft failure: 30.8%	NA	Recurrence: Graft rejection: Graft failure: Microperforation: High IOP: Secondary glaucoma: Cataract: Wound dehiscence:	Full-bed DLK PK 10.3% 20.6% 0.0% 41.3% 1.7% 22.22% 13.8% 0.0% 6.9% 38.1% 3.4% 4.8% 3.4% 32.6% 3.4% 7.9%	Recurrence: 23% Rejection: 0% Inflammation: 7.6%

ACV: acyclovir; BCVA: best corrected visual acuity; CF: counting fingers; DALK: deep anterior lamellar keratoplasty; Full-bed DLK: Full-bed deep lamellar keratoplasty; HM: hand movement; HSK: Herpes Simplex Keratitis; IOP: intraocular pressure; LP: light perception; m: months; m: months; NA: Not applicable; PK: penetrating keratoplasty; postop: postoperative; preop: preoperative; pts: patients; VA: visual acuity

## QUALITY ASSESSMENT

The assessment of study quality and the risk for bias is shown in the Table 1. Overall, 5 studies were classified as moderate (22, 24, 30–32) in global rating, that means that the risk of bias is also moderate. On the other hand, 7 records had weak (21, 23, 25–29) global rating and high risk of bias.

## DISCUSSION

The present systematic review compared PK and DALK techniques in patients suffering from HSK-related corneal scarring. For the better understanding of our findings, the different available surgical procedures should be analyzed. PK is a full-thickness transplant procedure, in which a full-thickness resection of the patient's cornea is followed by transplantation of a full-thickness donor corneal graft. On the other hand, in DALK, host tissue is removed down to the DM and transplantation of a donor cornea is applied, following the removal of the donor endothelium (34). PK is indicated when all corneal layers (epithelium, stroma and endothelium) are affected. Thus, it seems that HSK cases which are involving the endothelium and are more severe would have more adverse outcomes than the less severe, not involving the endothelium HSK cases which are treated by DALK.

Our study outcomes indicate that both surgical approaches resulted in comparable improvement in VA. A direct comparison of the VA improvement between the two surgery groups was difficult to achieve because of the heterogeneity of the outcomes presentation. However, DALK demonstrated fewer complications in almost all reports except for one by Lyall et al. (27). A possible explanation of Lyall et al. for the increased rate of post-DALK complications was the fact that they used only 800 mg/day of ACV. Graft failure or graft melting has also been associated with low dose of ACV, but also with the non-use of topical steroids and antibiotics, in the report of Li S. et al. (22). In fact, further to ACV, the increased rates of graft survival following DALK are attributed to the intact recipient's endothelium, which assumes function almost immediately following the surgical procedure. The recovery of the function of the endothelium is facilitated by local cortisone drops. In general, in 9 out of 10 studies examining DALK complications (21–22, 24–26, 28–29, 31–32), the graft rejection rate was between 0% and 23.07%, while in one study (27), graft rejection occurred in 50% of DALK cases in a 56-month follow-up period. On the other hand, in the three studies examining PK complications (24–25, 30), the rate of graft rejection ranged between 0% and 41.3%.

To the best of our knowledge, this is the first review to report on PK and DALK following HSK-related scarring. Our outcomes suggest that, despite the fact, that both surgical interventions result in comparable improvement outcomes in VA, DALK is associated with fewer complications. Higher graft survival rates are correlated with higher ACV doses (above 800 mg/day), topical steroid and antibiotic drops.

Certain limitations of our study need to be noted. First, only few literature reports compared directly PK with DALK technique in HSK patients. As a consequence, many

of our results were indirectly derived from descriptive studies or studies whose main object was the comparison of patient groups based on other criteria. In addition, the literature reports showed a great heterogeneity in the way the outcomes, especially the VA, were presented. Therefore, the direct comparison of the outcomes was challenging. Moreover, we only included studies with immunocompetent subjects, so eventually a significant number of immunodeficient cases was excluded. Finally, most of the articles received a moderate or weak global rating in the quality assessment control, due to the fact that they were non-randomized reports. There is no doubt that the inherent difference between PK and DALK could be clearly specified by randomized controlled trials (RCTs) that would compare sufficiently powered sample of patients with the same HSK severity who would be divided in PK- and DALK-groups. However, this kind of studies would arise ethical issues since patients with less severe HSK would receive a more invasive treatment like PK, and patients with more severe HSK would undergo a less invasive operation like DALK. Since PK is applied in more severe HSK cases than DALK, but also the visual outcomes of these surgical approaches are equally good, someone could suggest that PK is a better procedure compared to DALK. However, these surgical approaches were difficult to be practically compared as the final VA is not the only criterion for a successful operation. In fact, other criteria should be considered such as failure rate, peri- and postoperative complications, and recurrence of herpetic keratitis in the graft. In addition to that, the two procedures have different indications as the DALK cannot be used when the DM and/or the endothelium have been compromised.

## CONCLUSIONS

In terms of postoperative visual acuity, both PK and DALK demonstrate comparable efficacy. DALK, which is applied in less severe HSK cases, is associated with fewer complications and better graft survival rates. High dosages of ACV, topical steroids and antibiotics contribute significantly to improved postoperative outcomes.

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# Development of Pharyngocutaneous Fistula after Total Laryngectomy: The Predictive Value of C-reactive Protein/Albumin Ratio

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## ABSTRACT

**Background:** We aimed to evaluate whether C-reactive protein(CRP)/ Albumin ratio (CAR) performed in the early postoperative period after total laryngectomy could be a predictive factor for the development of pharyngocutaneous fistula (PCF).

**Methods:** The files of patients with laryngeal squamous cell carcinoma who underwent total laryngectomy between January 2005 and January 2019 were retrospectively reviewed. Patients were divided into two groups: patients with PCF (PCF group) and without (Non-PCF group). CAR values and risk factors were compared between groups.

**Results:** The overall incidence of PCF was 23.2%. There was a statistically significant difference between the two groups in terms of CRP and CAR levels ( $p = 0.001$ ). The CAR value of 27.05 (sensitivity = 75.0% , specificity 68.2%, area under curve (AUC) = 0.742, 95% confidence interval 0.616–0.868) was determined as a cutoff value to describe the development of fistula in the early postoperative period. In multiple linear regression analysis, there was an independent relationship between presence of PCF and previous RT and CAR value.

**Conclusions:** CAR, performed in the early postoperative period, may be a new and useful marker for predicting PCF after total laryngectomy.

## KEYWORDS

total laryngectomy; pharyngocutaneous fistula; crp/albumin ratio

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## INTRODUCTION

Total laryngectomy (TL), still has critical importance in the treatment of laryngeal cancer. Although the number of operations performed for laryngeal and hypopharyngeal cancer has decreased after 1990s due to the recent advances in survival rates with chemotherapy and radiotherapy, TL provides a good local and regional control, especially in advanced stage patients (1, 2). The pharyngocutaneous fistula (PCF) can be defined as dissociation of the pharynx mucosa and resulting in salivary leakage to the skin (3, 4). PCF is the most common complication after TL and its management requires experience. PCF is the main reason for increased morbidity, delayed initiation of adjuvant therapy, prolonged hospital stay, increased treatment costs, and significantly reduces quality of life (4). Therefore, prevention of PCF or early diagnosis may reduce morbidity and mortality of patients. There may also be disagreements among the authors about the most important risk factors for the occurrence of this complication (5). However, patients can be categorized for PCF risk considering the factors related to the patient, tumor and treatment.

C-reactive protein (CRP)/Albumin Ratio (CAR), has been reported to be a new inflammatory prognostic marker (6). It is stated that CAR is a new independent prognostic factor for total survival and disease-free survival in many cancer types such as larynx, hypopharynx, bladder, hypopharynx and stomach cancer (6–9). In addition, CAR has been reported to be an independent and significant risk factor for postoperative complications at some surgical procedures (10). In this study, we investigated whether CAR performed in the early postoperative period could be a predictive factor for the development of PCF.

## MATERIALS AND METHODS

The files of patients with laryngeal squamous cell carcinoma who underwent TL surgery between January 2005 and January 2019 were retrospectively reviewed. Patients were divided into two groups: patients with PCF (PCF group) and without (Non-PCF group). Age, sex, alcohol and usage of cigarette, additional disease (hypertension, diabetes mellitus, chronic obstructive pulmonary disease), history of preoperative radiotherapy (RT) and tracheotomy, need for blood transfusion, tumor localization (supraglottic, glottic, subglottic), data on the tumor characteristics (T,N,M Staging, AJCC Stage) (11), status of extralaryngeal extension, postoperative hemoglobin (Hb), white blood cell (WBC), neutrophil/lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR), CRP, albumin, CAR value and American Society of Anesthesiologists (ASA) classification of patients were recorded. The blood parameters of the patients between the postoperative 2–4 days were considered in this study. The hematologic values were measured with in the first 20 minutes after venous puncture (bloodskeep in potassium EDTA tubes) and then analysed using Sysmex XP-300 (Sysmex Corporation, Japan). The NLR value was calculated by dividing the neutrophil count to the lymphocyte count, and the PLR by dividing the platelet count to the lymphocyte count. CAR was obtained by dividing the

serum CRP level (mg/L) by serum albumin (g/dL). TL patients with and without PCF were compared in terms of these parameters.

All surgical procedures were performed by two experienced surgeons or under their supervision. After TL, pharyngeal closure was performed with T-shaped closure technic using 3/0 vicryl (polyglactin) in all cases. Patients who underwent reconstruction with a flap after laryngectomy were excluded from the study. Each patient was fed on the first postoperative day with a nasogastric tube. All patients were fed with the same food supplement in accordance with their weight. In the postoperative period, patients were given intravenous ampicillin sulbactam for 1 week. PCF was diagnosed by revealing the saliva content around the stoma in the postoperative period. Blood values after PCF development were not recorded. None of the patients receiving preoperative RT treatment received concurrent chemotherapy. Ethical consent was obtained from the ethics committee of local university with the number 2019/1897 for this study.

## STATISTICAL ANALYSIS

Descriptive statistics were used to compare the general characteristics of all participants. Test of Normality, including Kolmogorov-Smirnov and Shapiro-Wilk tests, was used to determine the distribution of data. The data with normal distribution were given as mean  $\pm$  standard deviation. Categorical variables were shown as number (n). The comparison of the numerical data between groups was performed with the appropriate test from Independent Samples T test, Mann-Whitney test, ANOVA and Kruskal-Wallis test. Chi-square and Fisher's exact test were used to compare categorical variables. The receiver operating characteristic (ROC) curve analysis was used to determine the sensitivity and specificity values of CAR in determining PCF development after TL. Statistical Package for Social Sciences (SPSS) Windows software (ver. 22; IBM SPSS, Chicago, USA) was used for all statistical analyses. P value less than 0.05 was considered as statistically significant.

## RESULTS

A total of 86 patients were included in this study. There were 20 patients in the PCF group and 66 in the non-PCF group. The overall incidence of PCF was 23.2%. The mean age of the PCF group was 62.5 and the non-PCF group was 57.6. All of the PCF group consisted of male patients and there were 61 male and 5 female in the non-PCF group. There was no significant difference between the two groups in terms of age and gender. Five patients in the PCF group and 2 patients in the non-PCF group received preoperative RT. There was a significant difference between the groups in terms of preoperative RT treatment ( $p = 0.007$ ). There was no significant difference between the two groups in terms of preoperative tracheotomy, need for blood transfusion and additional disease ( $p > 0.05$ ) (Table 1). The patient who underwent TL due to the T<sub>2</sub> tumor was operated for recurrence after RT.



When we evaluated the blood parameters, there was no statistically significant difference between the groups in terms of Hb, WBC, PLR and albumin levels ( $p > 0.05$ ). There was a significant difference between the two groups in terms of GRP, NLR and CAR levels ( $p = 0.001$ ). CRP, NLR and CAR levels were significantly higher in the PCF group (Table 1) The CAR value of 27.05 (sensitivity = 75.0%, specificity 68.2%, area under curve (AUC) = 0.742, 95% confidence interval 0.616–0.868) was determined as a cutoff value to describe the development of PCF in the early postoperative period (Figure 1). In other words, patients with CAR > 27.05 in the early postoperative period had a higher risk of PCF than patients with a CAR < 27.05.

There was no significant difference between the PCF group and the non-PCF group in terms of tumor location, TNM classification, disease stage, extralaryngeal extension and ASA score ( $p > 0.05$ ) (Table 2). There was a significant difference between the two groups in terms of preoperative RT status and the number of patients who received RT treatment was higher in the PCF group. Additionally, in multiple linear regression analysis, we found an independent relationship between presence of PCF and previous RT (beta: 0.303,  $p: 0.003$ ) and CAR value (beta: 0.246,  $p: 0.014$ ) (Table 3).

**Tab. 1** Distribution of risk factors according to the patient groups.

Parameters	Patients with PCF (n = 20) ± sd	Patients without PCF (n = 66) ± sd	P value
Age	62.5 ± 9.9	57.6 ± 12.0	0.096
Gender (F/M)	0/20	5/61	0.257
BMI (kg/m)	22.5 ± 2.11	22.1 ± 1.43	0.668
Smoking	20	65	0.767
Alcohol	2	0	0.052
Previous RT	5	2	<b>0.007</b>
Cardiovascular Disease	3	8	0.496
COPD	4	13	0.601
Preoperative Tracheotomy	2	1	0.134
Blood Transfusion	1	15	0.064
Hb (g/dL)	13.2 ± 2.0	13.8 ± 1.8	0.194
WBC (10 <sup>3</sup> /mm <sup>3</sup> )	10.2 ± 2.6	10.5 ± 4.1	0.631
CRP (mg/l)	111.7 ± 45.9	71.5 ± 28.2	<b>0.001</b>
Albumin (g/dL)	3.1 ± 0.5	3.1 ± 0.6	0.576
CAR	37.2 ± 19.1	23.1 ± 9.7	<b>0.001</b>
NLR	14.9 ± 5.4	12.3 ± 7.9	<b>0.013</b>
PLR	262.4 ± 89.5	256.1 ± 169.3	0.132

PCF: Pharyngocutaneous fistula, BMI: Body Mass Index, RT: Radiotherapy, COPD: Chronic Obstructive Pulmonary Disease, Hb: Hemoglobin, WBC: White Blood Cell, CRP: C-reactive Protein, CAR: C-reactive Protein/Albumin Ratio, NLR: Neutrophil/Lymphocyte Ratio, PLR: Platelet / Lymphocyte Ratio

**Tab. 2** Distribution of tumor parameters according to the patient groups.

Tumor Parameters	Patients with PCF (n = 20)	Patients without PCF (n = 66)	P value
Site	Supraglottic	2	8
	Glottic	1	7
	Subglottic	1	3
	Transglottic	16	48
T classification	T2	0	1
	T3	9	29
	T4	11	36
N classification	N0	13	33
	N1	2	16
	N2	1	15
	N3	4	2
M classification	M0	20	66
	M1	0	0
Stage	Stage 3	9	25
	Stage 4	11	41
Extralaryngeal spread	7	29	0.329
ASA	ASA2	9	11
	ASA3	24	42

T: Tumor, N: Node, M: Metastasis, ASA: American Society of Anesthesiologists

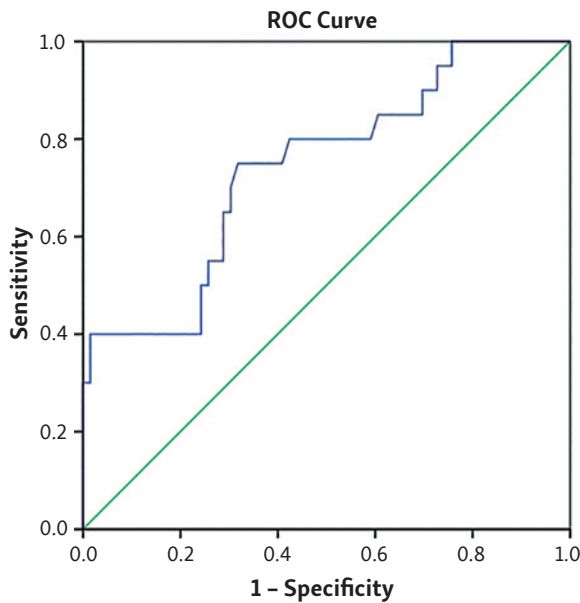
**Tab. 3** Multiple linear regression analysis.

Dependent variable	Independent variables	Beta regression coefficient	P value
Pharyngocutaneous fistula +	RT	0.303	<b>0.003</b>
	CAR	0.246	<b>0.014</b>

## DISCUSSION

PCF is a major complication that develops after TL and also a challenge for the surgeon and the patient. TL has become increasingly applicable as salvage procedure with the evolution of non-surgical organ protection protocols in the treatment of larynx and hypopharyngeal squamous cell carcinomas (12). Therefore, in future it is inevitable for head and neck surgeons to deal with more risky patients in terms of wound complications and PCF development. However, Suzana et al. (5) reported that the incidence of PCF was between 5% and 65% in the 70s and 80s, and between 9% and 25% in the last decade.

Although many studies have been conducted on PCF development after TL, there is still no consensus at this topic. However, there are many studies conducted to determine the most important risk factors. In a review, the most important risk factors for the development of PCF were preoperative and postoperative Hb <12.5 g/dL, pre and postoperative albumin <3.7 g/L, additional diseases, previous RT and chemoradiotherapy, long surgery time,



**Fig. 1** Receiver operating characteristic (ROC) curve for the development of PCF (sensitivity = 75.0%, specificity 68.2%, area under curve (AUC) = 0.742, 95% confidence interval 0.616–0.868).

blood transfusion during surgery, and inexperience of the surgeon (5). Paydarfar and Birkmeyer (13), reported that postoperative Hb <12.5 g/dL, previous tracheotomy, preoperative RT and preoperative RT combined neck dissection were associated with increased risk of PCF at their meta-analysis. Erdağ et al. (14) reported that control of concurrent systemic disease, preservation of hematological values in the pre- and post-operative period, ensuring adequate nutrition, and preference of erythrocyte suspensions for transfusion are key points in the prevention of PCF development. In addition to these traditionally specified risk factors, it is stated that wound classification, reconstruction with free flap compared to primary closure and ASA classification are among the important risk factors (15, 16). Dedivitis et al. (17) reported that, except for the mentioned risks, advanced primary tumor, performance status of patient are the important risk factors.

We evaluated our patients considering the risk factors mentioned above. 23.2% of our TL patients developed PCF. The main risk factor between the PCF and non-PCF group was previous RT. Although the main significant difference between the two groups was RT, it was observed that smoking was common in both groups, almost all of the patients were in advanced stage and most of the tumors were transglottic. It was previously reported that the absolute risk of PCF development after TL in patients receiving RT for laryngeal SCC mainly depends on the characteristics and localization of the primary tumor. In addition, while in patients with primary glottic T1 or T2 tumors, the absolute risk of PCF development was 11%, it was reported to increase to 35% at T3 or T4 extralaryngeal tumors (18).

In this study, we investigated whether CAR, NLR and PLR could be a predictive factor for early (postoperative 2–4 days) detection of PCF development after TL. Proinflammatory cytokines increase due to surgical injury, leading to changes in circulating acute phase proteins such as

CRP and albumin (19). Postoperative CRP levels are known to be a marker to predict postoperative inflammation and complications (20). Although albumin is associated with chronic diseases and nutritional deficiencies, it behaves like a negative acute phase protein and further decreases in response to surgical stress (21, 22). CAR is based on two circulating acute phase protein levels and is associated with surgery-induced inflammation. In addition, CAR is used to identify patients with a high probability of postoperative complications and it is superior to CRP alone in the prediction of postoperative complications (10). Xia-long Ge et al. (10) stated that CAR predicts postoperative results in patients undergoing elective colorectal surgery. They stated that combining CRP and albumin within the single index may be more accurate than CRP alone. In another study, CAR was claimed to be a potential predictor of anastomotic leakage in patients undergoing esophagectomy (23). Aires et al. (24) reported that patients with a NLR value of 2.5 and above had a higher risk of developing postoperative pharyngocutaneous fistula after TL. In our study, while there was a significant difference in NLR values between the two groups, there was no significant difference in terms of PLR. Also, we did not detect any relationship between these two markers and PCF development in the regression analysis.

In this study, CAR was significantly higher in the PCF group than the non-PCF group. We also found that patients with CAR > 27.05 had a higher risk of PCF than patients with a CAR < 27.05. Furthermore, there was an independent relationship between presence of PCF and previous RT and CAR value in multiple linear regression analysis. Perhaps CAR value can give us an idea about need to early compressive dressing or necessary to wait for oral feeding due to the possible PCF. When we examined the groups, we found out that the main difference between the two groups was previous RT. There was no significant difference between the groups in terms of other clinical risk factors. However, serum albumin levels of both groups were below 3.5 g/dL which is described as risky level in the literature. The difference between the groups in terms of CAR value was due to the difference in CRP levels rather than the albumin. The role of both albumin and CRP is extensively known in development of PCF. But as we said before, CRP is a positive acute phase reactant and albumin is a negative phase reactant. In the event of a possible PCF, the serum levels of these two markers (CRP, albumin) can change in the opposite direction, therefore combination of these two markers can give more sensitive information about PCF development than the changes alone in the blood levels of these two markers. But probably due to the low number of patients, we could not achieve results that would fully support our hypothesis in this study. Nevertheless, with this study, we wanted to draw attention to the fact that CAR may have a role in the development of PCF after TL and we wanted to inspire the studies that can be carried out with many more patient numbers at this topic.

This study has some limitations. First of all, the retrospective nature of this study may have caused to the exclusion of some patients without detailed medical records. Although the study groups were similar in terms of risk factors in general, there was a difference between

the groups in terms of previous RT, which is one of the main risk factors of PCF development. Therefore, if this study may be performed in patients with primary TL, more healthy outcomes could be obtained. In addition, only patients who underwent TL with the diagnosis of laryngeal SCC were included in the study, patients with hypopharynx SCC were not included. Another limitation of this study is the lack of CAR values of patients at the preoperative period.

## CONCLUSION

This is the first study to examine the relationship between PCF development and CAR in TL patients. CAR, performed in the early postoperative period, may be a new and useful marker for predicting PCF after TL. However, role of CAR at PCF after TL should be supported by studies with a higher number of patients in order to make more accurate comments on this subject. Especially in high-risk patients, we think that correlating it with other blood parameters can be more useful.

## FINANCIAL DISCLOSURE

The authors declare that this study has received no financial support. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

This study have been approved by the ethics committee of Necmettin Erbakan University with the number 2019/1897 and have been performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

An informed consent was supplied from the participants.

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# Sensitization to Molecular Components in 104 Atopic Dermatitis Patients in Relation to Subgroups of Patients Suffering from Bronchial Asthma and Allergic Rhinitis

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## ABSTRACT

**Background:** Atopic dermatitis (AD) is a chronic inflammatory skin disease. The progression from AD to bronchial asthma (AB) and allergic rhinitis (AR) is called atopic march. The aim of this study was to evaluate the difference in the sensitization to molecular components in patients suffering from AD in relation to subgroups of patients with AR and AB.

**Material and Methods:** The complete dermatological and allergological examinations were performed. Specific IgE antibodies against 112 molecular components were measured with the multiplex ImmnoCAP ISAC test.

**Results:** Altogether 104 atopic dermatitis patients (50 men, 54 women) at the average age 40.1 years were examined. The sensitization to molecular components was confirmed in 93.3% of patients. The sensitization to components of mites, grasses, trees, animals, moulds, and shrimps was significantly more frequent in patients with severe form of AD and the sensitization to components of grasses, trees, and moulds was significantly higher in subgroup of patients with AB. In subgroup of patients suffering from AR the higher occurrence of pollen-derived and pollen-food derived PR-10 proteins, grasses, mites, and animals was observed also.

**Conclusions:** We have confirmed the significant differences in the sensitization to molecular components in patients suffering from severe form of AD, and in subgroups of patients suffering from AB and AR. These molecular components may play the important role in the consecutive development of different allergy pathologies called atopic march.

## KEYWORDS

molecular components; multiplex ISAC testing; severity of atopic dermatitis; bronchial asthma; allergic rhinitis; atopic march

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## INTRODUCTION

Atopic dermatitis (AD) is a chronic inflammatory skin disease. The pathogenesis of AD involves susceptibility genes, immune dysregulation, and disrupted epidermal barrier function resulting in increased transepidermal water loss (TEWL), permeation of irritants, microbes and aeroallergens (1–3). The immune response is polarized towards innate immunity cells, such as dendritic cells, innate lymphoid cells type 2 (ILC-2), mast cells, basophilic granulocytes, and eosinophilic granulocytes. The direct contact of skin with allergens could trigger signals to initiate Th2 allergic response. A typical manifestation of allergic inflammation is the production of IgE antibodies directed against causative allergens (4, 5). A progression from AD to allergic rhinitis (AR) and bronchial asthma (AB) may develop in the first several years of life. This process is a phenomenon called atopic march (6). Positive correlations have been demonstrated between the severity of AD and the risk of development bronchial asthma, and allergic rhinitis (1). However, the exact mechanism explaining the atopic march remains to be elucidated. Emerging data suggest that epithelial cell-derived cytokines such as thymic stromal lymphopoietin (TSLP), IL-25, and IL-33 may drive the progression from AD to bronchial asthma and food allergy (1). Various allergens may cause exacerbation of eczematous skin lesions in atopic dermatitis. The main allergenic sources are food, moulds, trees, weeds, grasses, mites, and animals (7). Specific IgE sensitization to food and aeroallergens, especially to house dust mites, pollen-derived and plant-derived food allergens has been described in adult AD patients (8).

Diagnostic tests of allergic diseases such as *in-vivo* skin prick tests or *in-vitro* measurement of specific IgE, and basophil activation test, are based on allergens derived from natural sources (extracts). Each allergen source is a very complex mixture of allergenic and non-allergenic proteins. This methodology has its limitations. Allergic extracts are incapable to differentiate between primary sensitization and immunological cross-reactivity (9–11). Progress in laboratory diagnostics of IgE-mediated allergy was made by the introduction of component-resolved diagnosis (CRD). The molecularly defined allergens (components) are used in a singleplex test or a multiplex allergen microarray assay. The main goal of CRD is to distinguish between the mainly species-specific components and the cross-reactive allergen molecules. It is evident that CRD enhances the specificity of IgE-diagnosis in polysensitized respiratory allergies (12), and can be also applied in food allergies (13) and atopic dermatitis (13, 14) and in addition, may reveal unexplained anaphylaxis (10).

The aim of this study was to evaluate the sensitization to molecular components in relation to severity of AD and to determine whether there are some differences between the sensitization profiles in subgroups of patients suffering from bronchial asthma and allergic rhinitis. To identify the sensitization and co-sensitization to species-specific and cross-reacting allergen components we used a commercially available microarray immunoassay ImmunoCAP ISAC. It is a complex assay for simultaneous determination of allergen specific IgE (sIgE) against 112 molecular

components (purified natural and recombinant) originating for more than 50 sources (14, 15). The major advantage of ISAC is the comprehensive IgE pattern obtained with a minute amount of serum (16).

Only few reports demonstrate the sensitization to molecular components in atopic dermatitis patients and the relation of this sensitization to the severity of atopic dermatitis, and to the occurrence of bronchial asthma and allergic rhinitis (8, 17, 18). It should be emphasized that in this study we focused on the degree of sensitization only and not on its clinical relevance.

## MATERIAL AND METHODS

### PATIENTS

In the period 2018–2019, 104 patients suffering from atopic dermatitis were examined. All these patients were examined at the Department of Dermatology and Venereology, University Hospital Hradec Králové, Czech Republic. Complete dermatological and allergological examination was performed in all patients enrolled to this study. The diagnosis of atopic dermatitis was made using the Hanifin-Rajka criteria (19). Exclusion criteria were long term therapy with cyclosporin or systemic corticoids, pregnancy, breastfeeding. Patients with atopic dermatitis having other systemic diseases were excluded from the study as well. This study was approved by the Ethics Committee, University Hospital Hradec Králové, Czech Republic.

### BRONCHIAL ASTHMA

The diagnosis of bronchial asthma (AB), was determined according to the guidelines of the Global Initiative for Asthma (GINA) at allergy outpatients clinic of the Institute of Clinical Immunology and Allergology, University Hospital Hradec Králové, Czech Republic.

### ALLERGIC RHINITIS

The evaluation of allergic rhinitis (AR), was made according to the allergy testing and personal history.

### SEVERITY OF ATOPIC DERMATITIS

Severity of atopic dermatitis was scored according to SCORAD index (Scoring of Atopic Dermatitis) with the assessment of topography items (affected skin area), intensity criteria and subjective parameters (20). The severity of atopic dermatitis was evaluated with SCORAD index as a mild form to 25 points, as a moderate form over 25 to 50 points, as a severe form over 50 points. The evaluation of the severity was calculated as the average SCORAD measured every 2 month during 1 last year (21).

### EXAMINATION OF SPECIFIC IGE TO MOLECULAR COMPONENTS BY IMMUNOCAP ISAC TEST

Samples of blood were collected from the cubital vein. Blood serum was isolated by centrifugation and stored under  $-70^{\circ}\text{C}$  until analysis. Repeated thawing and freezing

were avoided. The levels of specific IgE in all patients were determined by the component-resolved diagnosis microarray-based sIgE detection assay ImmunoCAP ISAC sIgE 112 (Phadia, Thermo Fisher Scientific, Uppsala, Sweden). ImmunoCAP ISAC sIgE 112 is a solid-phase semi-quantitative multiple immunoassay which enables to determine 112 different components from 51 allergen sources (22, 23). The molecular components are applied in triplicates (70 recombinant, 42 purified natural) to ensure the test reproducibility. The specific IgE values are measured in arbitrary units ISU-E (ISAC Standardized Units), measuring range of 0.3–100 ISU-E. The results of sIgE are presented semi-quantitatively in 4 classes: < 0.3 ISU-E negative, 0.3 > 0.9 ISU-E low positivity, 0.9 > 15 ISU-E moderate positivity, ≥ 15 ISU-E very high positivity (the level of specific IgE greater than 0.3 ISU-E was considered as positive) (14). The analysis was conducted according to the manufacturer's instruction.

### STATISTICAL ANALYSIS

We analysed the data to determine whether the occurrence of sensitization to examined molecular components is in relation to the severity of atopic dermatitis. In addition, we assess if there are some differences in the sensitization to molecular components in the subgroups of patients suffering from bronchial asthma or allergic rhinitis. Relative frequencies of sensitization to the investigated molecular components were determined in all patients according to the severity of atopic dermatitis, bronchial asthma and allergic rhinitis. Pairs of these categories were enrolled in the contingency tables and the Chi-square independence test was performed. The significance level was set to 5%.

### RESULTS

We examined 104 patients suffering from AD, 50 men and 54 women with the average age 40.1 years (s.d. 15.9) and with the average SCORAD index 39 points (s.d. 13.1). Mild form of AD was recorded in 13.5% of patients, moderate

**Tab. 1** The characteristics of patients.

Number of patients with AD	104 patients (50 men, 54 women)
age	average age 40.1 years (s.d. 15.9)
index SCORAD	average SCORAD 39 points (s.d. 13.1)
sensitization to allergen components	97 patients (93.3%)
mild form of AD	14 patients (13.5%)
moderate form of AD	61 patients (58.7%)
severe form of AD	29 patients (27.9%)
subgroups of patients:	
number of patients with AB	58 patients (55.8%)
number of patients with AR	79 patients (76.0%)

AD – atopic dermatitis, AB – bronchial asthma, AR – allergic rhinitis

form of AD in 58.7% of patients and severe form of AD in 27.9% of patients. Subgroup of patients suffering from bronchial asthma or allergic rhinitis was recorded in 55.8% and 76.0%, respectively. The sensitization to at least one of the tested molecular components was confirmed in 93.3% of patients. No positive results to molecular components were obtained in 6.7% patients. The characteristics of the patients are summarised in Table 1. The results describing the sensitization patterns to tested components in all AD patients are listed below and shown in Table 2.

In the whole group of patients, the highest sensitization rate was observed to pollen-derived components and *Betulaceae*-specific components. Timothy is present on the biochip in eight molecular components. Sensitization rate to rPhl p 1 (61.0%) was followed by nPhl p 4 (52.0%), rPhl p 5 (43.0%), rPhl p 6 (42.0%), rPhl p 2 (39.0%) and rPhl p 11 (20.0%). The sensitization rate to polcalcin rPhl p 7 and profilin rPhl p 12 was lower than 10.0%. The second most frequent sensitization was 57.0% to rBet v 1, which was followed by other *Betulaceae*-specific components, such as rCor a 1.0101 (45.0%) and rAln g 1 (43.0%). Sensitization to pollen-food derived PR-10 proteins was observed frequently as well; on the other hand, sensitization to profilin rBet v 2 and polcalcin rBet v 4 were observed rarely (< 10%). The sensitization rates to mite-specific molecules were observed more frequently in the group 2 (rDer p 2, 46.0% and rDer f 2, 45.0%) in comparison with group 1 (nDer p 1, 36.0% and nDer f 1, 34.0%). The sensitization to animal components was observed most frequently to cat allergen rFel d 1 (42.0%) and dog allergens rCan f 1 (39.0%) and rCan f 5 (26.0%), which were followed by the sensitization to animal lipocalins rFel d 4 (29.0%), rEqu c 1 (27.0%), nMus m 1 (20.0%), rCan f 2 (17.0%). The frequency of sensitization to individual components is shown in Table 2 and schematically illustrated in Figure related to Table 2.

### SENSITIZATION TO THE MOLECULAR COMPONENTS IN RELATION TO THE SEVERITY OF ATOPIC DERMATITIS

All 104 patients were divided into three groups according to SCORAD index. We evaluated the relative frequency of positive reactions to molecular components in patients with mild, moderate and severe form of atopic dermatitis. Increased relative frequency of positive reactions ranging from mild to moderate to severe form of AD was confirmed for most molecular components. Positive results of specific IgE antibodies against 47 molecular components were presented in mild form of AD, and 105 components were recorded in moderate and severe form of AD.

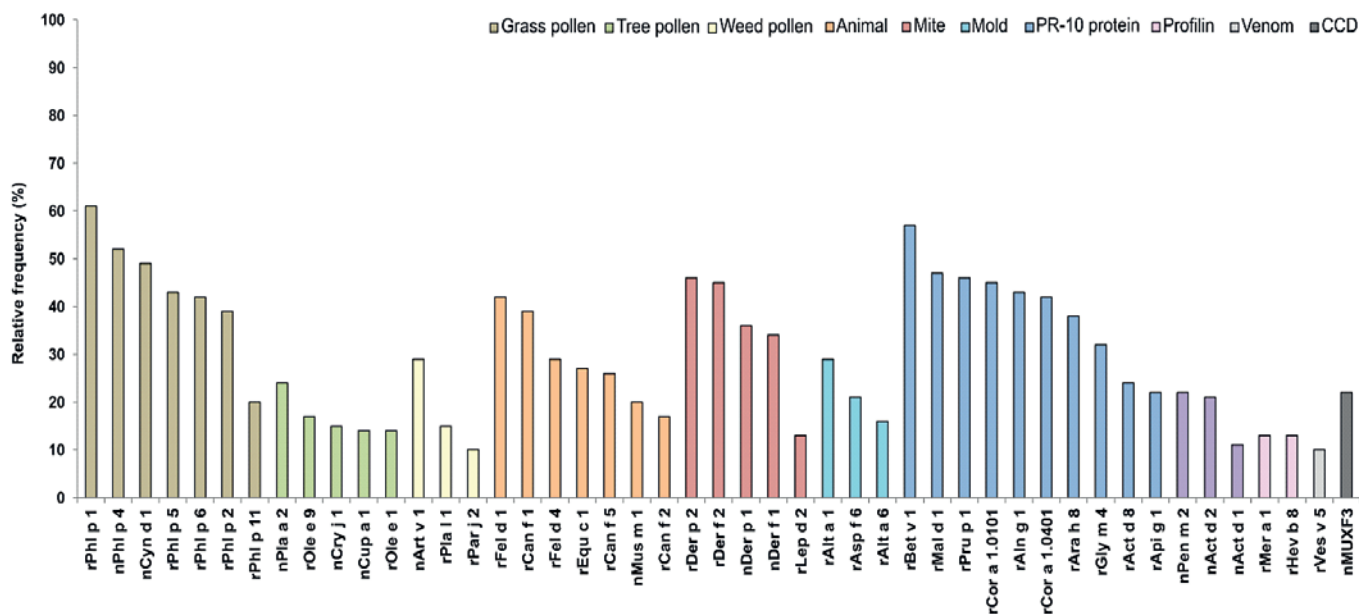
In the severe form of AD (29 patients; 100%) the highest sensitization rate to grass-species specific component rPhl p 1 (timothy, beta-expansin) reached 72.4% of patients. The second most frequent sensitization rate to components of mites rDer f 2 and rDer p 2 (NPC2 family) was observed in 65.5% of patients with severe form of AD.

The relation between the occurrence of sensitization to some molecular components and the severity of AD was confirmed. The following molecular components were recorded significantly more frequently in patients with severe form of AD than with mild form of AD ( $p < 0.05$ ).

**Tab. 2** The list of molecular components according to positivity (relative frequency) in 104 patients with atopic dermatitis.

Allergen source	Molecular components	Protein groups	No. of patients (%)
Timothy grass	<b>rPhl p 1</b>	β-expansin	61.0
Birch	<b>rBet v 1</b>	PR-10 protein	57.0
Timothy grass	<i>nPhl p 4</i>	Berberine bridge enzyme	52.0
Bermuda grass	<b>nCyn d 1</b>	β-expansin	49.0
Apple	<b>rMal d 1</b>	PR-10 protein	47.0
House dust mite	<b>rDer p 2</b>	NPC2 family	46.0
Peach	<b>rPru p 1</b>	PR-10 protein	46.0
Hazel pollen	<b>rCor a 1.0101</b>	PR-10 protein	45.0
House dust mite	<b>rDer f 2</b>	NPC2 family	45.0
Timothy grass	<b>rPhl p 5</b>	Ribonucleases	43.0
Alder	<b>rAln g 1</b>	PR-10 protein	43.0
Timothy grass	<i>rPhl p 6</i>	Grass group 6	42.0
Cat	<b>rFel d 1</b>	Uteroglobin	42.0
Hazelnut	<b>rCor a 1.0401</b>	PR-10 protein	42.0
Timothy grass	<i>rPhl p 2</i>	Expansin	39.0
Dog	<b>rCan f 1</b>	Lipocalin	39.0
Peanut	<b>rAra h 8</b>	PR-10 protein	38.0
House dust mite	<b>nDer p 1</b>	Cysteine protease	36.0
House dust mite	<b>nDer f 1</b>	Cysteine protease	34.0
Soy	<b>rGly m 4</b>	PR-10 protein	32.0
Mugwort	<b>nArt v 1</b>	Defensin	29.0
<i>Alternaria</i>	<b>rAlt a 1</b>	Acidic glycoprotein	29.0
Cat	<b>rFel d 4</b>	Lipocalin	29.0
Horse	<b>rEqu c 1</b>	Lipocalin	27.0
Dog	<b>rCan f 5</b>	Arginine esterase, Prostatic kallikrein	26.0
Plane tree	<b>nPla a 2</b>	Polygalacturonase	24.0
Kiwifruit	<b>rAct d 8</b>	PR-10 protein	24.0
Shrimp	<i>nPen m 2</i>	Arginine kinase	22.0
Celery	<b>rApi g 1</b>	PR-10 protein	22.0
CCD	<i>nMUXF3</i>	Sugar epitope from bromelain	22.0
<i>Aspergillus</i>	<i>rAsp f 6</i>	Mn superoxide dismutase	21.0
Kiwifruit	<b>nAct d 2</b>	Thaumatococcus-like protein	21.0
Timothy grass	<i>rPhl p 11</i>	Ole e 1-related protein	20.0
Mouse	<b>nMus m 1</b>	Lipocalin	20.0
Olive pollen	<b>rOle e 9</b>	1.3-β-glucanase	17.0
Dog	<i>rCan f 2</i>	Lipocalin	17.0
<i>Alternaria</i>	<i>rAlt a 6</i>	Enolase	16.0
Japanese cedar	<b>nCry j 1</b>	Pectate lyase	15.0
Plantain	<b>rPla l 1</b>	Ole e 1-related protein	15.0
Cypress	<b>nCup a 1</b>	Pectate lyase	14.0
Olive pollen	<b>rOle e 1</b>	Common olive group 1	14.0
Annual mercury	<i>rMer a 1</i>	Profilin	13.0
Storage mite	<i>rLep d 2</i>	NPC2 family	13.0
Latex	<i>rHev b 8</i>	Profilin	13.0
Kiwifruit	<b>nAct d 1</b>	Cysteine protease	11.0
Wall pelitory	<b>rPar j 2</b>	Lipid transfer protein	10.0
Yellow jacket	<b>rVes v 5</b>	Antigen 5	10.0

CCD – cross-reactive carbohydrate determinants; major allergens are highlighted in bold (e.g. **rPhl p 1**), minor allergens are highlighted in italics (e.g. *rPhl p 6*) and cross-reactive components are illustrate in grey box (e.g. **rBet v 1**); molecular components with sensitization rate less than 10% are not mentioned



**Fig. 1 related to Tab. 2:** Sensitization rate to molecular components according to positivity (relative frequency) in 104 patients with atopic dermatitis. Molecular components are sorted by protein group and decreasing relative frequency in each group; molecular components with sensitization rate less than 10% are not mentioned.

High sensitization rate of positive reactions were reported against the major inhalant allergen components of grasses rPhl p 1 (timothy, beta-expansin); mites rDer f 2 and rDer p 2 (house dust mite, NPC2 family); animals rCan f 1

(dog, lipocalin), rCan f 5 (dog, arginine esterase), rFel d 1 (cat, uteroglobulin), rFel d 4 (cat, lipocalin), rEqu c 1 (horse, lipocalin), nMus m 1 (mouse, lipocalin); and vegetables rApi g 1 (celery, PR-10 protein) in severe form of AD. The

**Tab. 3** The list of molecular components according to positivity (relative frequency) in mild, moderate and severe form of AD – statistically significant difference (p-value < 0.05).

Allergen source	Molecular components	No. of patients in mild form of AD (%)	No. of patients in moderate form of AD (%)	No. of patients in severe form of AD (%)	p-value
		(14 patients = 100%)	(61 patients = 100%)	(29 patients = 100%)	
Timothy grass	<b>rPhl p 1</b>	4 (28.6%)	40 (65.6%)	21 (72.4%)	0.015
House dust mite	<b>rDer f 2</b>	2 (14.3%)	26 (42.6%)	19 (65.5%)	0.006
House dust mite	<b>rDer p 2</b>	2 (14.3%)	27 (44.3%)	19 (65.5%)	0.006
Cat	<b>rFel d 1</b>	2 (14.3%)	27 (44.3%)	17 (58.6%)	0.023
Cat	<b>rFel d 4</b>	0 (0.0%)	14 (23.0%)	16 (55.2%)	0.000
Dog	<b>rCan f 1</b>	0 (0.0%)	27 (44.3%)	13 (44.8%)	0.006
Dog	<b>rCan f 5</b>	1 (7.1%)	14 (23.0%)	12 (41.4%)	0.040
Horse	<b>rEqu c 1</b>	0 (0.0%)	14 (23.0%)	15 (51.7%)	0.001
Mouse	<b>nMus m 1</b>	0 (0.0%)	10 (16.4%)	11 (37.9%)	0.008
Olive	<b>rOle e 9</b>	0 (0.0%)	10 (16.4%)	9 (31.0%)	0.040
Birch	<i>rBet v 2</i>	0 (0.0%)	3 (4.9%)	6 (20.7%)	0.021
<i>Alternaria</i>	<i>rAlt a 6</i>	0 (0.0%)	7 (11.5%)	10 (34.5%)	0.005
<i>Aspergillus</i>	<i>rAsp f 6</i>	0 (0.0%)	11 (18.0%)	12 (41.4%)	0.004
Shrimp	<i>nPen m 2</i>	1 (7.1%)	7 (11.5%)	14 (48.3%)	0.000
Celery	<b>rApi g 1</b>	0 (0.0%)	12 (19.7%)	10 (34.5%)	0.031
Yellow jacket	<b>rVes v 5</b>	0 (0.0%)	3 (4.9%)	7 (24.1%)	0.006
Walnut	<b>nJug r 2</b>	0 (0.0%)	3 (4.9%)	6 (20.7%)	0.021
Egg white	<b>nGal d 2</b>	0 (0.0%)	0 (0.0%)	5 (17.2%)	0.001
Peanut	<b>rAra h 1</b>	0 (0.0%)	1 (1.6%)	4 (13.8%)	0.028
Wheat	<b>nTri a a_TI</b>	0 (0.0%)	0 (0.0%)	3 (10.3%)	0.018

AD – atopic dermatitis; major allergens are highlighted in bold (e.g. **rPhl p 1**), minor allergens are highlighted in italics (e.g. *rAlt a 6*), and cross-reactive components are illustrate in grey box (e.g. **rApi g 1**)



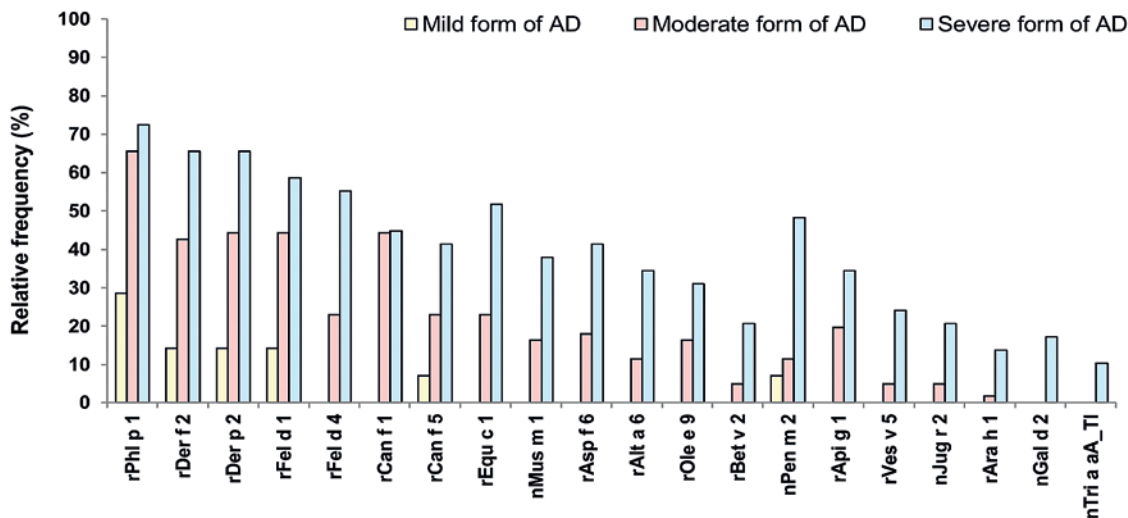


Fig. 2 related to Tab. 3: Sensitization to molecular components according to positivity (relative frequency) in mild, moderate and severe forms of AD – statistically significant difference (p-value < 0.05).

sensitization rate to minor allergen components of moulds rAlt a 6 (*Alternaria*, enolase), rAsp f 6 (*Aspergillus*, Mn superoxide dismutase), and crustaceans nPen m 2 (shrimp, arginine kinase), was also high. Furthermore, significant differences were confirmed, but with a lower frequency of positive cases, against to tree pollen allergens, such as major component of olive rOle e 9 (glucanase) and to minor component of birch rBet v 2 (profilin) in severe form of AD. Moreover, the lower frequency of positive cases were observed in the major food allergens, such as nTri a aA\_TI (wheat, trypsin/ $\alpha$ -amylase inhibitor), rAra h 1 (peanut, 7S globulin), nJug r 2 (walnut, 7S globulin), nGal d 2 (egg white, ovalbumin), and the major allergen of wasp rVes v 5 (yellow jacket, antigen 5). These differences (p-value < 0.05) are shown in Table 3 and schematically illustrated in Figure related to Table 3.

#### SENSITIZATION TO THE MOLECULAR COMPONENTS IN RELATION TO SUBGROUPS OF PATIENTS SUFFERING FROM ALLERGIC RHINITIS AND BRONCHIAL ASTHMA

We determined whether there are some differences between the sensitization to molecular components in relation to concomitant bronchial asthma or allergic rhinitis in all of 104 atopic dermatitis patients.

The occurrence of AB was recorded in 55.8% of patients. Following molecular components were observed significantly more frequently in patients with AB, such as a minor grass-specific component rPhl p 2 (timothy, expansin), and a major component of trees rOle e 9 (olive, glucanase). Sensitization rate to the minor component of mould rAlt a 6 (*Alternaria*, enolase) was also high. Moreover, lower frequency of positive case against the major allergen of wasp rVes v 5 (antigen 5) was noticed in patients with AB. Interestingly, the sensitization rate to polcalcin regarding rPhl p 7 (timothy) and rBet v 4 (birch) showed no positive results of sIgE in patients with AB. Surprisingly, the occurrence of the CCD component MUXF3 were observed more frequently in subgroup of patients with

AB. These differences (p-value < 0.05) are shown in Table 4 and schematically illustrated in Figure related to Table 4.

The occurrence of AR was recorded in 76.0% of patients. These molecular components were observed significantly more frequently in patients with AR: major components of pollen-derived and pollen-food derived PR-10 proteins (Bet v 1 family), such as rBet v 1 (birch), rCor a 1.0101 (hazel), rMal d 1 (apple), rPru p 1 (peach), and rApi g 1 (celery). Sensitization rate to the major grass-specific components nCyn d 1 (bermuda grass, beta-expansin), rPhl p 5 (timothy, ribonuclease), and a minor component rPhl p 6 (timothy, grass group 6), and a major component of house dust mite rDer f 2 (NPC2 family) and lipocalins, such as rCan f 1 (dog), rFel d 4 (cat), rEqu c 1 (horse), nMus m 1 (mouse) was also high in patients with AR. The sensitization rate to nAct d 1 (kiwifruit, cysteine protease) was less frequent. These differences (p-value < 0.05) are shown in Table 5 and schematically illustrated in Figure related to Table 5.

#### DISCUSSION

Skin barrier abnormalities have been proposed to play an essential role in the initiation of atopic dermatitis in infancy (6). Epicutaneous allergens sensitization through an impaired skin barrier stimulates antigen-presenting cells and induces Th2 responses and consequent allergic manifestations. In a Th2-promoting environment, T-cell/B-cell interactions in regional lymph nodes lead to an excessive IgE switch (1). Simultaneous release of memory T cells into the circulation and their homing back to the skin can induce not only exacerbation of AD but also can initiate the atopic march. The progression of atopic disorders from AD in infants to allergic rhinitis and asthma in children is usually described as atopic march. The most important factor that precipitates atopic march is now considered an impaired epidermal barrier. Barrier disturbances result from genetic defects and early epicutaneous sensitization to food and aeroallergens may be enhanced by damage of the skin barrier function (6, 24). However, the exact

**Tab. 4** The list of molecular components according to positivity (relative frequency) in subgroup of patients suffering from bronchial asthma – statistically significant difference (p-value < 0.05).

Allergen source	Molecular components	No. of patients without AB (%)	No. of patients with AB (%)	p-value
		(46 patients = 100%)	(58 patients = 100%)	
Timothy grass	<i>rPhl p 2</i>	13 (28.3%)	28 (48.3%)	0.038
Olive	<b>rOle e 9</b>	4 (8.7%)	15 (25.9%)	0.024
<i>Alternaria</i>	<i>rAlt a 6</i>	2 (4.3%)	15 (25.9%)	0.003
CCD	nMUXF3	6 (13.0%)	17 (29.3%)	0.047
Yellow jacket	<b>rVes v 5</b>	1 (2.2%)	9 (15.5%)	0.022
Birch	<i>rBet v 4</i>	4 (8.7%)	0 (0.0%)	0.022
Timothy grass	<i>rPhl p 7</i>	7 (15.2%)	0 (0.0%)	0.002

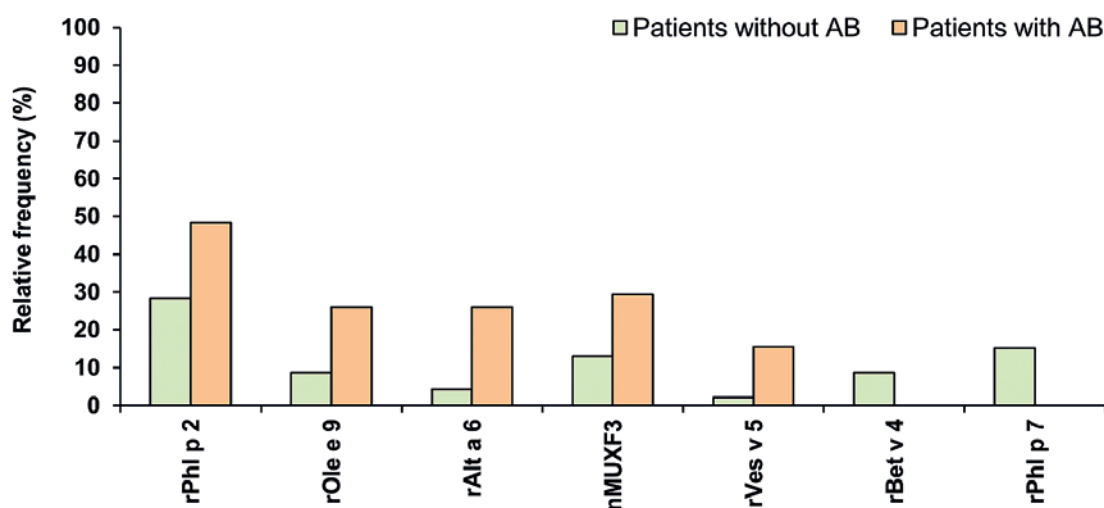
AB – bronchial asthma, CCD – cross-reactive carbohydrate determinants; major allergens are highlighted in bold (e.g. **rOle e 9**), minor allergens are highlighted in italics (e.g. *rAlt a 6*), and cross-reactive components are illustrate in grey box (e.g. *rBet v 4*)

mechanisms explaining the atopic march remain to be elucidated.

Progress in laboratory diagnostics of IgE-mediated allergies is the use of component-resolved diagnosis that implies determination of sIgE against purified native and recombinant components which are used in laboratory as singleplex or multiplex assays (25). There is currently no consensus on the use of multiplex microarray ImmunoCAP ISAC worldwide (26–28). Hatzler et al. (29) investigated the IgE response to grass-specific pollen allergens and determined that sensitization can start years before clinical disease onset through the process called “molecular spreading”. There is some evidence (30, 31) that studies show a strong correlation between results of extract-based skin prick testing (SPT), multiplex microarray assay (ImmunoCAP ISAC, Phadia) and fluorescence enzyme immunoassays (UniCAP, Phadia) with excellent correlation especially in pollen allergens (32) and house dust mite allergens (33). Molecular allergy diagnosis may improve the risk evaluation, sorts out genuine from cross-reactive sensitizations, and finally, improves the accuracy of allergen immunotherapy indication. Currently, more than 130 molecular components are available for *in-vitro* sIgE

testing which can be performed on singleplex or multiplex measurement platforms (e.g. for ALEX<sup>2</sup> more than 170 components) (5). In the WAO-ARIA-GA2LEN consensus document (5) molecular-based allergy diagnosis is recommended in the third line-diagnostic workup, if medical history and exact-based skin prick- and sIgE testing are inconclusive. Multiplex assays are especially suited for use in patients with complex sensitization patterns or symptoms, in small children with limited skin area, in elderly when skin test is less reliable, and when medications interfering with skin prick testing cannot be discontinued (5, 34).

We compared our results with other studies from the Middle-European region in the point of view the outcomes describing the sensitization patterns to molecular components (9, 35, 36). Panzner et al. investigated 1255 sensitized patients, with a mean age of 29 years, and with the following diagnoses: chronic rhinitis (73%), bronchial asthma (41%), atopic dermatitis (34%), urticaria or edema (19%), and/or anaphylaxis (11%) (35, 36). In our study, we investigated the group of patients suffering from atopic dermatitis, some of these patients suffer from bronchial asthma (55.8%) and from allergic rhinitis (76.0%). Our



**Fig. 3 related to Tab. 4:** Sensitization to molecular components according to positivity (relative frequency) in subgroup of patients suffering from bronchial asthma – statistically significant difference (p-value < 0.05).

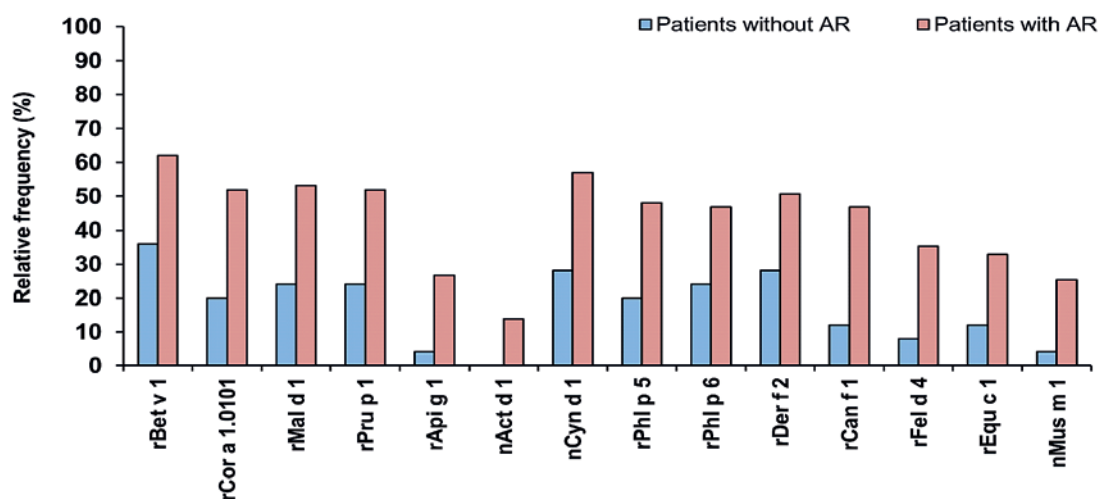
**Tab. 5** The list of molecular components according to positivity (relative frequency) in subgroup of patients suffering from allergic rhinitis – statistically significant difference (p-value < 0.05).

Allergen source	Molecular components	No. of patients without AR (%)	No. of patients with AR (%)	p-value
		(25 patients = 100 %)	(79 patients = 100 %)	
Birch	<b>rBet v 1</b>	9 (36.0 %)	49 (62.0 %)	0.022
Hazel pollen	<b>rCor a 1.0101</b>	5 (20.0 %)	41 (51.9 %)	0.005
Apple	<b>rMal d 1</b>	6 (24.0 %)	42 (53.2 %)	0.011
Peach	<b>rPru p 1</b>	6 (24.0 %)	41 (51.9 %)	0.015
Celery	<b>rApi g 1</b>	1 (4.0 %)	21 (26.6 %)	0.016
Kiwifruit	<b>nAct d 1</b>	0 (0.0 %)	11 (13.9 %)	0.048
Bermuda grass	<b>nCyn d 1</b>	7 (28.0 %)	45 (57.0 %)	0.012
Timothy grass	<b>rPhl p 5</b>	5 (20.0 %)	38 (48.1 %)	0.013
Timothy grass	<i>rPhl p 6</i>	6 (24.0 %)	37 (46.8 %)	0.043
House dust mite	<b>rDer f 2</b>	7 (28.0 %)	40 (50.6 %)	0.048
Dog	<b>rCan f 1</b>	3 (12.0 %)	37 (46.8 %)	0.002
Cat	<b>rFel d 4</b>	2 (8.0 %)	28 (35.4 %)	0.008
Horse	<b>rEqu c 1</b>	3 (12.0 %)	26 (32.9 %)	0.042
Mouse	<b>nMus m 1</b>	1 (4.0 %)	20 (25.3 %)	0.021

AR – allergic rhinitis; major allergens are highlighted in bold (e.g. **rBet v 1**), minor allergens are highlighted in italics (e.g. *rPhl p 6*), and cross-reactive components are illustrate in grey box (e.g. **rMal d 1**)

results are in agreement with the Panzner's hypothesis (9) that grasses (*rPhl p 1*) and *Betulaceae* (*rBet v 1*) components comprised the vast majority of pollen sensitizations in the condition of Middle-European region. On the other hand, the sensitization to animal allergen molecules was higher in our study (to *rFel d 1* in 42.0%, to *rCan f 1* in 39.0%, to *rFel d 4* in 29.0%, to *rEqu c 1* in 27%, to *rCan f 5* in 26%); the sensitization to mite molecular allergens was in our study higher also (to *rDer p 2* in 46.0%, to *rDer f 2* in 45%, to *nDer p 1* in 36.0%, to *nDer f 1* in 34.0%). In the Panzner's study, the sensitization rate to animal allergen molecules was confirmed to *rFel d 1* in 31.8%, to *rCan f 1* in 13.9%, to *rCan f 5* in 16.4%, to *rEqu c 1* in 6.2%, to *rFel d 4* in 5.3% (36). The sensitization to at least one mite-specific molecule (*nDer p 1*, *rDer p 2*, *nDer f 1*, *rDer f 2*) was observed in 32.7% of

patients in Panzner's study (35). The explanation of higher sensitization rate to animal and mite molecular allergens in our group of patients can be in the fact, that we included patients suffering from atopic dermatitis; in the Panzner's study, atopic dermatitis patients represent only 34% of patients. Our results may demonstrate the significance of disturbed epidermal barrier, resulting in increased transepidermal water loss and permeation of allergens, irritants, and microbes. It is evident that the direct contact of skin with allergens could trigger signals to initiate Th2 allergic response. Emerging data now suggest that epithelial cell-derived cytokines such as TSLP, IL-33, and IL-25 may drive the progression from atopic dermatitis to bronchial asthma and food allergy (1–3). In 2014, it was reported that IgE antibodies to *Der p 11* are more common in

**Fig. 4 related to Tab. 5:** Sensitization to molecular components according to positivity (relative frequency) in subgroup of patients suffering from allergic rhinitis – statistically significant difference (p-value < 0.05).

sera from patients with atopic dermatitis (37). Thus, sensitization to this allergen may reflect the fact that the eczematous skin allows easy penetration of allergens even with molecular weight as high as 100,000. In ISAC testing, the molecular component Der p 11 is not present, so we cannot compare it with our results. Although the house dust mite allergens are present in the mite bodies, the main allergenic sources are the mite faeces which, with a diameter higher than 10 µm (37), can be easily inhaled into the airways and consequently be entered deep into the lungs (37).

Our preliminary results regarding the analysis of sensitization to molecular components in atopic dermatitis patients were already published or they are in press (38, 39, 40, 41). We analysed the data to find the molecular components with the highest underlying probability of sensitization in patients suffering from atopic dermatitis and in subgroups of patients with allergic rhinitis and bronchial asthma (38). According to our results, the order of molecular components in mild form of AD is not statistically significant, but a set of molecular components with the highest underlying probability in moderate and severe form of AD and in a subgroup of patients suffering from allergic rhinitis was recorded (38). According to the statistical method with cluster analysis, we found 10 clusters with different numbers of molecular components (39). Fundamental position have the components rPhl p 1 (timothy), rBet v 1 (birch), rAlt a 1 (*Alternaria*) followed by molecular components of NPC2 family, cysteine protease, tropomyosin, uteroglobin, lipocalin and PR-10 protein. Our results correspond to the association of molecular components into protein families according to their biochemical structure (39). The preliminary data regarding the sensitization to molecular components in 81 atopic dermatitis patients were processed in other publications (40, 41).

There are various allergens that can trigger an eczema flare up. An allergen-specific IgE-mediated response to a wide spectrum of food and inhalant allergens, especially house dust mite, pollen and plant-derived food allergens, has been described in adult AD patients (8). The aim of our study was to identify some differences in the occurrence of the sensitization to the molecular components in the group of 104 atopic dermatitis patients in relation to severity of AD and to the occurrence of bronchial asthma and allergic rhinitis.

According to our results, rPhl p 1 is a leading molecular component in patients suffering from severe form of AD as well as in subgroups of patients with AR and AB. However, the occurrence of rPhl p 1 was significantly more frequent only in patients with severe form of AD. The IgE response usually evolves from monosensitization to polysensitization, this phenomenon has been described as “molecular spreading”. rPhl p 1 (beta-expansin) is a presumable the “initiator” of the sensitization process in most patients. It is the major grass-specific allergen belongs to grass group 1. In addition, it is an essential diagnostic marker for allergic patients to establish “true sensitization” to grass pollen (timothy). In a few cases the grass pollen allergy might be evoke by isolated IgE sensitization to another major grass-specific allergen (e.g. rPhl p 5), but it is rather unlikely (34). nPhl p 4 is a minor grass-specific allergen, a highly glycosylated protein, that can bind to IgE specific

for cross-reactive carbohydrate determinants (CCDs) (5). High sensitization rate to nPhl p 4 was observed in our study in subgroup of patients with AR, but there was no evidence of significant difference in comparison to patients without AR. The major allergen of bermuda grass pollen is a nCyn d 1 from the beta-expansin family that is commonly found in subtropical regions but is not presented in our region (rarely in south Moravia) (9). Possible cross-reactivity with beta-expansins from other grasses, especially with the major allergen of timothy could be the explanation of higher occurrence of nCyn d 1 in subgroup of AR patients in our study. Sensitization to other components from the same pollen source usually come before the sensitization to panallergens (e.g. polcalcins and profilins) which are typically recognized at the late stage of molecular spreading (29, 42). Specific IgE to rPhl p 7 determine a relatively distinct category of grass pollen allergic patients, who may suffer from more severe symptoms, with a higher prevalence of bronchial asthma, and a higher frequency of other allergic comorbidities (34). In contrast to these findings, our subgroup of patients with bronchial asthma showed no positive results of sIgE against polcalcins rPhl p 7 and rBet v 4 ( $p < 0.05$ ).

Surprisingly, not negligible sensitization rate to component of olive rOle e 9 was observed more frequently in patients with severe form of AD and in subgroup of patients with AB; rOle e 9 (glucanase) is a major olive allergen which commonly cause sensitivity in geographical areas exposed to high levels of olive pollen (43). Moreover, rOle e 9 shares some common epitopes with glucanases from birch and ash pollens, tomato, potato, pepper, banana, and latex (44) that might be the explanation of higher sensitization rate to this component.

Our results pointed out that the sensitization to major components of mites in severe form of AD might be associated with the sensitization to major components of animals (rCan f 1, rCan f 5, rFel d 1, rFel d 4, rEqu c 1, nMus m 1) and minor components of moulds (rAlt a 6, rAsp f 6). Animals are the second most important source of indoor allergens after house dust mites (45). They are considered as risk factors for the development of allergic rhinitis and asthma (46). Numbers of dog, cat, and horse allergens have been described. Vast majority belongs to the protein families of uteroglobin, lipocalin and kallikrein. We observed the high sensitization rates to lipocalins and uteroglobins. Lipocalins represent the most important protein family, which are synthesized in salivary glands. Most of them are major animal allergens (rCan f 1, rFel d 4, rEqu c 1, nMus m 1). Can f 5, considered as a major dog allergen, is a prostatic kallikrein (arginine esterase) that is found only in male dogs (34, 47). Fel d 1, a major cat allergen, is a uteroglobin expressed in salivary glands and skin. The severity of induced symptoms varies widely and cat and dog allergy could be the principal risk factor of both rhinitis and asthma, associated with higher severity, which can develop into a life-threatening condition (45, 47). It is in an agreement with our results. House dust mites (HDM) belong to the most potent indoor allergen sources that are associated with allergic manifestations in the respiratory tract and the skin (37). The effect of mites on the human organism is complex because of mites can carry microbial and fungal

antigens, respectively pathogen-associated molecular patterns (PAMPs), thus initiating mechanisms of innate immunity. The largest number of HDM molecules is known in the two most important species *Dermatophagoides (D.) farinae* and *D. pteronyssinus* (34). Their molecular components can be divided into groups according to protein families. Group 1 (cysteine proteases) includes the major molecular components of nDer f 1 and nDer p 1, which show 85% homology (35). Group 2 (NPC2 family) comprise the major components rDer f 2 and rDer p 2, which show up to 90% homology within the group (35). These allergens are assumed to be the specific components for mite allergy. The presence of sIgE to major molecular components nDer p 1, rDer p 2 (34) and Der p 23 (48), that are present in fecal particles of mites, has strong association with asthma. However, this is not in the concordance with our results. We recorded that the sensitization to group 2 allergens was significantly higher only in patients with severe form of AD (rDer f 2 and rDer p 2) and in subgroup of patients suffering from allergic rhinitis (rDer f 2). Recently identified Der p 11 is present predominantly in the muscle of HDM bodies that belong to the family of proteins known as paramyosins. Der p 11 seems to be a useful serological marker for HDM-allergic patients suffering from atopic dermatitis (37). Unfortunately, molecular components Der p 23 and Der p 11 are not included in the ISAC test, thus we cannot compare them with our results. A strong immunogenic potential of mite components may play a crucial role in the atopic march (49). Moreover, an impaired epidermal barrier is considered as the most important factor that elicit atopic march (50). The prevalence of mould sensitization displays wide geographical variability (34). Considering the sensitization to minor components rAsp f 6 (*Aspergillus fumigatus*, Mn superoxide dismutase) and rAlt a 6 (*Alternaria alternata*, enolase) were recorded with significantly higher occurrence in patients suffering from severe form of AD (rAsp f 6, rAlt a 6) and in subgroup of patients with bronchial asthma (rAlt a 6). Sensitization to *Alternaria (A.) alternata* is a risk factor to develop asthma (51). Furthermore, bronchial asthma is characterized by more persistent symptoms and enhanced disease severity. *A. alternata* is a widespread saprophyte that is usually found in outdoor, however, it can also occur in indoor environments. Moreover, sensitization to *A. alternata* seems to be a triggering factor in the development of poly-sensitization (52). Nevertheless, the clinical relevance of high level of specific IgE to *Alternaria* in patients with AD remains unclear (53). *Aspergillus (A.) fumigatus* is a mould permanently present in the indoor and outdoor environment (34). *Aspergillus* allergy is rare in atopic individuals without asthma or cystic fibrosis (54). Interestingly, the phylogenetically highly conserved allergens Asp f 6, Asp f 8, Asp f 11, Asp f 27, Asp f 28 and Asp f 29 show a high degree of cross-reactivity with other mould proteins belonging to the same families. The clinical relevance of these reactions remains elusive (34).

Bet v 1 homologous allergens (PR-10 like proteins) shows a highly cross-reactivity pattern. Birch (rBet v 1), followed by alder (rAln g 1) and hazel (rCor a 1.0101) constitute the most potent cause of tree pollen allergy (55). According to our result, there is no relation between the

subgroup of patients with AB and the sensitization to *Betulaceae*-specific components. The significantly higher occurrence of sensitization to PR-10 proteins was recorded to rApi g 1 (celery) in patients suffering from severe form of AD and to major components of tree-specific components, such as rBet v 1 (birch), rCor a 1.0101 (hazel), and pollen-food derived proteins like rMal d 1 (apple), rRup p 1 (peach), and rApi g 1 (celery) in subgroup of patients suffering from AR. These pollen-food derived PR-10 proteins mainly cause local manifestations of allergic reactions and may induce a variety of “pollen-food” syndromes (8). Results of sensitization profile in the subgroup of patients with AR suggest that AR patients are mostly sensitized to inhalant allergens (pollen or pollen-food derived PR-10) via the respiratory tract or digestive system. Röckmann et al. (8) demonstrated that sensitization to food-derived PR-10 allergens occurred most frequently in AD patients but there was no association between their presence and severity of AD. They recorded higher sensitization rate to rAra h 1 (peanut) and nBos d lactoferrin (cow’s milk) in patients with severe form of AD. We recorded in the higher frequency the presence of specific IgE to major food allergens of wheat (nTri a aA\_T1), egg white (nGal d 2), walnut (nJug r 2) and peanut (rAra h 1) but only in patients with severe form of AD. The outcome of our analysis could be influenced by the fact that some allergens showed zero frequencies of positive values which were particularly evident when we have compared mild, moderate and severe form of AD. The molecular component nTri a aA\_T1 is a part of the ISAC assay and it is a trypsin/ $\alpha$ -amylase inhibitor of wheat grains. Its designation is not based on the official WHO/IUIS database ([www.allergen.org](http://www.allergen.org)), but it names from Phadia. Peanut allergens are the most common trigger of food-induced anaphylaxis (34). rAra h 1 (7S globulin) is a thermostable seed storage protein whose allergenicity can be increased by roasting (34). nGal d 2 (ovalbumin), a major allergen, is the most abundant egg white protein. It is less heat-stable than ovomucoid (nGal d 1). IgE responses to Gal d 2 indicate a risk for clinically relevant reaction to raw or slightly heated egg (34, 56). nJug r 2, a highly glycosylated protein, has been identified as an important allergen in common walnut. Native vicilin-like protein nJug r 2 (7S globulin), can bind to IgE specific for cross-reactive carbohydrate determinants (CCDs), and can also be raised in patients sensitized to CCDs (57). For this reason, the real clinical significance of a positive nJug r 2 result must be carefully evaluated in the context of the results of other components and clinical findings (5).

Food allergy to shellfish (crustaceans and molluscs) may cause cross-sensitization and clinical reactivity to house dust mites, insects and arachnids (34). Numbers of crustacean allergens have been described. Tropomyosin is a major shrimp allergen. Several others allergenic components have been identified (arginine kinase, myosin light chain and sarcoplasmic calcium binding protein) (34). Our result showed the higher occurrence of a minor shrimp allergen nPen m 2 (arginine kinase) in patients suffering from severe form of AD. Similar to tropomyosin, arginine kinase is highly abundant in invertebrate muscle, and up to now it has been described in various shellfish and other invertebrates such as mites, cockroaches, crab, shrimp,

and crayfish (58, 59). Unlike tropomyosins, they show ter-molabile properties (60).

Component-resolved diagnostics seems to be a promising tool for the diagnosis of food allergy, offering the potential to determine specific phenotypes and estimate the risk of immune response to a given allergen. Nevertheless, the diagnostic accuracy of these laboratory tests varies across studies. Therefore, their clinical utility remains unclear (61). The assessment of diagnostic accuracy (sensitivity, specificity) for certain food allergen components (ISAC test) will be the topic for our future research.

## CONCLUSIONS

Sensitization to following molecular components of grasses (rPhl p 1), trees (rOle e 9, rBet v 2), house dust mites (rDer f 2, rDer p 2), animals (rFel d 1, rFel d 4, rCan f 1, rCan f 5, rEqu c 1, nMus m 1), moulds (rAlt a 6, rAsp f 6), and foods (nTri a aA\_TI, nGal d 2, rAra h 1, nJug r 2, nPen m 2, rApi g 1) was significantly more frequent in patients with severe form of AD. In this regard, we recommend enrolling the assessment of the presence of specific IgE against these components into the clinical procedures and treatment of allergy patients with the special respect to the risk of development of severe reactions.

Typically, in subgroup of patients suffering from allergic rhinitis the significantly higher sensitization to molecular components, such as tree pollen (rBet v 1, rCor a 1.0101), grass pollen (rPhl p 5, rPhl p 6), house dust mites (rDer f 2), animals (rCan f 1, rFel d 4, rEqu c 1, nMus m 1), and foods (rMal d 1, rPru p 1, rApi g 1, nAct d 1) is recorded in our study. In patients with bronchial asthma the significantly higher sensitization to molecular components of grasses (rPhl p 2), trees (rOle e 9) and *Alternaria* (rAlt a 6) was recorded. These molecular components may play the important role in the atopic march in an individual patient.

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## CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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# Safety and Efficacy of Using Tranexamic Acid at the Beginning of Robotic-Assisted Radical Prostatectomy in a Double-Blind Prospective Randomized Pilot Study

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## ABSTRACT

**Background:** The prophylactic administration of tranexamic acid has been shown to be appropriate for procedures with a high risk of perioperative bleeding in cardiac surgery and orthopaedics. In urology the ambiguous results have been reported. Our goal was to evaluate the effect of tranexamic acid administration in robotic-assisted radical prostatectomy (RARP). A pilot, prospective, double-blind, randomized study was conducted to evaluate this effect.

**Methods:** The study included 100 patients who received RARP in the period from April 2017 to January 2018. The patients were randomly assigned to study and control groups of 50 patients each.

**Results:** The median follow-up was 6 months. Lower haemoglobin level drop weighted for gram of operated prostate was observed in the study group when treating the dorsal vein complex (DVC) at the beginning of the procedure ( $p = 0.004$  after 3 hours and  $p < 0.001$  after 24 hours). There was no evidence of any serious side effect of tranexamic acid.

**Conclusion:** We demonstrated the safety of tranexamic acid at RARP. In addition, we showed that administration of tranexamic acid at the beginning of RARP significantly reduces the decrease in haemoglobin after the procedure when treating the DVC at the beginning of the procedure.

## KEYWORDS

tranexamic acid; robotic-assisted radical prostatectomy; prostate carcinoma

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## INTRODUCTION

Prostate adenocarcinoma is the most common malignancy in men. The incidence increases over time and with patient age. Prostate adenocarcinoma is the second most common cause of death due to malignancy in men, after lung cancer. In patients with moderate- and low-risk prostate cancer and life expectancy more than 10 years, the method of choice is radical prostatectomy or radiotherapy (1–3).

In recent years, we have seen a general tendency towards minimally invasive surgical procedures. In the treatment of localized prostate cancer, laparoscopic and robotic-assisted radical prostatectomy (RARP) have become the gold standard. Despite tremendous development in the technology and technique of robotic-assisted radical prostatectomy over more than 25 years, we still need to look for ways to improve oncological and functional outcomes (4–8).

We can assume that lower perioperative blood loss will lead to a better view in the operating field and improve the outcomes. Decreasing postoperative blood loss may lead to faster recovery after the procedure (9). Concerns have been raised about the possible relationship between the administration of blood derivatives and an increased risk of relapse of malignancy and tumour-specific mortality (10, 11).

Tranexamic acid is an antifibrinolytic used to relieve bleeding. The mechanism of action lies in binding to plasma free plasminogen with higher affinity than tissue plasminogen activator. It prevents its conversion to plasmin, which is responsible for the degradation of fibrin polymers. The result is greater stability of the fibrin clot at the site of bleeding and, therefore, lower blood loss (12–14). Use of tranexamic acid during or after the operation does not improve results, unlike administration prior to surgery. A biological explanation is that tranexamic acid may bind plasminogen in the early phase of the fibrinolytic cascade, after the beginning of the procedure, reducing tissue plasminogen activator activity up to 80% (15).

In urological surgery, increased conversion of plasminogen to plasmin should occur, both by washing the tissue plasminogen activator from the destroyed tissue and by urokinase present in the urine (16). Only a few studies have been published to date on the use of tranexamic acid in transurethral prostate resection, open radical prostatectomy, and open radical cystectomy. The first paper did not confirm the positive effect in terms of reduced perioperative and postoperative blood loss in prostate transurethral resection (17). Increasing evidence of the beneficial use of tranexamic acid in cardiac surgery, neurosurgery, traumatology, and orthopaedics has led to the renewal of this idea (18–20).

In urology, the results are not clear, as they differ in various urological procedures, e.g. endoscopic transurethral resections with negative results (21) to positive results in other procedures (22–25). Recent meta-analysis confirmed these positive results for cancer patients, in general (26).

Our goal was to investigate the effect of tranexamic acid on perioperative and postoperative blood loss, procedure duration, and reduction of the risk of positive surgical

margin in RARP. A secondary goal was to monitor adverse events, such as acute myocardial infarction, stroke, generalized convulsions, and thromboembolic events.

## MATERIAL AND METHODS

This pilot, prospective, randomized, double-blind, and placebo-controlled study was approved by the Ethics Committee in University Hospital in Hradec Králové (201704S06P). Between April 2017 and January 2018, 144 RARP were performed in our department.

We acquired patients scheduled for RARP without pelvic lymphadenectomy who provided informed consent. We primarily excluded patients who did not sign informed consent ( $n = 18$ ) or had pelvic lymphadenectomy planned ( $n = 15$ ). We assumed that pelvic lymphadenectomy would distort the results by prolonging the procedure time and increasing the volume of fluids in the suction and drain after surgery. Another exclusion criterion was coagulation disorder, congenital (e.g., Leiden mutation or thrombophilia) or iatrogenic due to the chronic use of antiaggregants or anticoagulants. We also excluded patients who had a thromboembolic, cerebral, or acute coronary event 6 months prior to prostatectomy ( $n = 9$ ). None of the patients had a proven allergic reaction to tranexamic acid, which was the last exclusion criterion. A total of 100 patients were finally analyzed in the study.

After enrolment, each patient was assigned a unique number from 0 to 100 generated by an independent worker at [www.randomizer.org](http://www.randomizer.org) prior to the study. The first 50 numbers generated represented patients assigned to the tranexamic acid group. The remaining numbers were assigned to the control group. The group assignment information was placed in a sealed envelope marked with the study number.

On the day of the procedure, an assigned nurse prepared an infusion set according to the information in the sealed envelope with the patient's study number. For patients in the treatment group, 1.5 g of tranexamic acid was added to 100 ml of physiological saline and was given within 5 minutes after the robotic system was docked.

The therapeutic concentration of tranexamic acid in plasma ranges from 5 mg/kg to 10 mg/kg. After an intravenous dose of 10 mg/kg, plasma concentration was maintained for 3 hours, but in orthopaedics, it was proved to be inadequate (27, 28). The 20 mg/kg dose maintains a therapeutic level for 8 hours but increases the risk of a thromboembolic event (19). Based on the above-mentioned literature, we decided to administer a single dose of 1.5 g tranexamic acid to all patients in the treatment group, corresponding to 10–20 mg/kg.

All patients underwent RARP without pelvic lymphadenectomy. A total of four surgeons performed the procedure using almost the same technique. The only difference was treatment of the dorsal vein complex (DVC). One surgeon sutured the DVC at the beginning of the procedure with two rounds of resorbable monofilament suture ( $n = 38$ ). Other surgeons treated the complex with several rounds of barbed suture after completing prostatectomy ( $n = 62$ ). To accelerate the return of continence, a modified

**Tab. 1** Cohort parameters (TA – tranexamic acid group).

	Age (years)		BMI		iPSA (ng/ml)		Specimen weight (g)	
	TA	Placebo	TA	Placebo	TA	Placebo	TA	Placebo
Average	64.3	65.3	26.5	28.5	6.8	6.8	61.3	55.4
SD	5.9	5.7	2.8	3.9	3.3	2.4	28.5	17.7
P value	p = 0.363		p = 0.396		p = 0.549		p = 0.549	

Rocco stitch was performed in all patients. The anastomosis was performed by two V-loc stitches, the ends of which were tied. Antibiotic prophylaxis was provided by a single dose of potent aminopenicillin as recommended by the antibiotic centre (n = 98); fluoroquinolone was administered in patients with an allergy to aminopenicillin (n = 2). During the procedure, the urinary tract opening time (from bladder opening to completion of urethro-vesical anastomosis), console time, amount of fluid in the suction system, and the weight of prostate were monitored.

The prophylactic dose of low molecular weight heparin (LMWH) was administered to every patient for minimum of 7 days, starting the day before the procedure.

Evaluating perioperative blood loss in RARP has several difficulties. The amount of aspirated fluid during the procedure is further distorted by the lymphatic secretions of the traumatized tissue, irrigation fluid and the production of urine in the open urinary tract. The volume of intravenous infusions was recorded. Due to an inability to accurately measure actual diuresis, hourly diuresis was determined arbitrarily for all patients at 50 ml per hour. The amount of urine produced was calculated based on the urinary tract opening time. The measurement of the suction fluid volume was done with an accuracy of 25 ml after aspiration of all residual irrigation fluid from reservoir at the end of the procedure. The amount of urine and irrigation fluid (500 ml) was subtracted from the amount of fluid in the suction capsule to estimate perioperative blood loss.

During the operation, we recorded console time, which represented the activity of the console surgeon from the connection of the robotic system to its disconnection. During this period, the urinary tract opening time was recorded from the opening of the bladder neck to the completion of the urethro-vesical anastomosis.

We assumed that the results would be significantly affected by the weight of the prostate. A larger prostate could mean longer operating time and greater blood loss. Therefore, all results were weighted for the grams of prostatic tissue.

Another factor that could have significantly affected the results was the patient's body mass index (BMI). Patients with higher BMI usually tend to not tolerate the Trendelenburg position as well as those with a lower BMI, a position that is essential for a good overview of the operating field. Obesity of the patients worsens the overview and increases the tendency for lymphorrhagia. BMI > 30 is also risk factor for thromboembolism.

The blood count was taken before the procedure, 3 hours after the procedure and the next day at 6 a.m. The

drainage fluid was monitored on postoperative day 1 POD1 and 2 and the total number of blood transfusions was recorded. A permanent urinary catheter was extracted on POD7. Intraabdominal drain was extracted on the POD (postoperative day) 1 or 2 if there were no complications. Only three patients had urinary leakage of the anastomosis, detected initially biochemically (creatinine level over 500 µmol/l) in drain and subsequently cystographically. In another two patients, increased lymphatic secretion from the drain was found. These patients were excluded from the evaluation of postoperative blood loss (n = 5).

Upon completion in 100 patients, the cases were unblinded and statistical processing performed, using NCSS statistical software (NCSS, Kaysville, UT, USA).

## RESULTS

The characteristics of the treatment and control groups were not significantly different. Differences between age and PSA were assessed by the nonparametric Mann-Whitney U test. BMI and specimen weight were compared by the Kolmogorov-Smirnov test (Table 1).

No difference was found between the two groups in the volume of infusions during the procedure (Table 2).

No difference was found between the two groups in perioperative blood loss and perioperative blood loss related to gram of operated prostate (Table 3).

Blood count before the procedure was taken by patients' general practitioner which might have biased the

**Tab. 2** Perioperative crystalloid infusions (ml).

	TA	Placebo
Average	1825	1770
SD	751	603
P-value	p = 0.656	

**Tab. 3** Perioperative blood loss.

	Overall blood loss (ml)		Blood loss per gram (ml/g)	
	TA	Placebo	TA	Placebo
Q1	93.0	97	1.99	1.94
Q2	222.5	212	3.58	3.92
Q3	377.5	386	6.61	7.80
P-value	p = 0.712		p = 0.480	

results. Three patients were not analyzed because the haemoglobin level was higher after the procedure than before (n = 2 from treatment group, n = 1 from control group). No significant difference was found in the absolute numbers for the haemoglobin decrease in 3 hours (3hr Hb-drop) and the day after surgery (24hr Hb-drop). Depending on the decrease in haemoglobin relative to gram of operated prostate, differences became significant (3hr Hb-drop/g and 24hr Hb-drop/g) (Table 4). The most striking differences between the groups were observed in patients with the DVC treated at the beginning of the procedure (Table 5).

In contrast, there were no significant differences between both groups if DVC was treated at the end of the procedure (Table 6).

Patients with biochemically and cystographically proven urinary leakage of anastomosis (n = 2 in treatment group, and n = 1 in control group) and patients with extensive lymphatic secretion (n = 2 in control group)

were excluded from the evaluation of drainage. A significant difference was found between the study and control groups in overall postoperative drainage volume and overall postoperative drainage volume weighted for grams of prostate (Table 7).

No significant differences in console time were found between the study and control groups. Effects of definitive histology findings worsening (up-staging and upgrading) (Table 8, 9) or operating procedure variations on console time have also not been demonstrated. The only factor that significantly influenced console time was the patient's BMI (Table 10).

We did not observe significant reduction in incidence of positive surgical margins in group with tranexamic acid.

All patients were followed for 3 months after the procedure. Postoperative complications were recorded and graded based on severity according to the Clavien-Dindo definition (29). Only one episode of brachial artery

**Tab. 4** Hemoglobin level drop overall.

	Initial Hb level (g/l)		3hr Hb-drop (g/l)		3hr Hb-drop / g (g/l/g)		24hr Hb-drop (g/l)		24hr Hb-drop / g (g/l/g)	
	TA	Placebo	TA	Placebo	TA	Placebo	TA	Placebo	TA	Placebo
	(n = 48)	(n = 49)	(n = 48)	(n = 49)	(n = 48)	(n = 49)	(n = 48)	(n = 49)	(n = 48)	(n = 49)
Average	149.5	151.6	15.1	16.7	0.30	0.32	22.5	24.3	0.44	0.47
SD	7.7	8.3	8.4	6.8	0.22	0.13	10.3	8.1	0.28	0.20
Q1	144.0	148.0	10.0	13.0	0.15	0.24	16.5	19.0	0.20	0.31
Q3	154.0	158.0	20.0	22.0	0.43	0.41	28.0	30.0	0.52	0.62
P-value			p = 0.190		p = 0.013		p = 0.154		p = 0.096	

**Tab. 5** Hemoglobin level drop in patients with DVC suturing at the beginning of procedure.

	Initial Hb level (g/l)		3hr Hb-drop (g/l)		3hr Hb-drop / g (g/l/g)		24hr Hb-drop (g/l)		24hr Hb-drop / g (g/l/g)	
	TA	Placebo	TA	Placebo	TA	Placebo	TA	Placebo	TA	Placebo
	(n = 17)	(n = 19)	(n = 17)	(n = 19)	(n = 17)	(n = 19)	(n = 17)	(n = 19)	(n = 17)	(n = 19)
Average	150.6	152.2	9.3	17.3	0.15	0.33	17.6	25.6	0.29	0.50
SD	8.7	4.5	6.9	7.3	0.12	0.13	7.6	7.7	0.12	0.18
Q1	149.0	149.0	5.0	12.5	0.07	0.21	11.0	21.0	0.19	0.38
Q3	154.0	156.0	12.0	22.8	0.18	0.42	22.0	31.8	0.35	0.65
P-value			p = 0.006		p = 0.004		p = 0.004		p = 0.001	

**Tab. 6** Hemoglobin level drop Hemoglobin without DVC suturing at the beginning of procedure.

	Initial Hb level (g/l)		3hr Hb-drop (g/l)		3hr Hb-drop / g (g/l/g)		24hr Hb-drop (g/l)		24hr Hb-drop / g (g/l/g)	
	TA	Placebo	TA	Placebo	TA	Placebo	TA	Placebo	TA	Placebo
	(n = 31)	(n = 31)	(n = 31)	(n = 31)	(n = 31)	(n = 31)	(n = 31)	(n = 31)	(n = 31)	(n = 31)
Average	149.0	151.0	17.9	15.9	0.36	0.31	24.7	22.9	0.49	0.45
SD	7.1	9.8	8.2	6.3	0.23	0.13	10.8	8.5	0.73	0.21
Q1	143.0	146.0	12.3	13.0	0.17	0.23	19.3	17.0	0.27	0.29
Q3	154.0	158.0	22.0	21.0	0.47	0.40	28.8	29.0	0.73	0.60
P-value			p = 0.657		p = 0.594		p = 0.807		p = 0.773	

**Tab. 7** Overall volume in drain.

	Drain volume (ml)		Drain volume / g (ml/g)	
	TA	Placebo	TA	Placebo
	(n = 48)	(n = 47)	(n = 48)	(n = 47)
Q1	6.25	50	0.12	0.75
Q2	50	80	0.81	1.37
Q3	100	100	2.03	2.50
P-value	p = 0.048		p = 0.023	

**Tab. 8** Gleason score (GS) incidence.

GS	Initial		Definitive	
	TA	Placebo	TA	Placebo
6	34	29	11	8
7a	13	15	24	30
7b	2	4	9	6
8	1	1	5	3
9	0	1	1	2

**Tab. 9** Prognostic factor worsening.

	TA	Placebo
Up-grading	32	26
Up-staging	12	5
Positive surgical margin (PSM)	11	14

thrombosis was recorded in a patient in the control group (Dindo/Clavien II). The patients receiving tranexamic acid did not experience any complications in terms of stroke, acute myocardial infarction, or thromboembolic disease.

Two cases of significant postoperative bleeding were noted. One patient in the control group had an approximately 500 ml hematoma in the pelvis, which was conservatively controlled, with no transfusion (Dindo/Clavien II). Another patient from the treatment group underwent acute laparoscopic revision for incipient haemodynamic instability, demonstrated haemoperitoneum, and a significant decrease in the blood count. During the revision,

a haemoperitoneum of 3000 ml was aspirated, but the source of bleeding was not found. Four blood transfusions were given (Dindo/Clavien IVa). No further complications were noted.

Two patients (one from the study group and the other from the control group) had increased secretion from drains, requiring longer drainage without further consequences (Dindo/Clavien I). In three patients with cystographically proven urinary leakage (one from the study group and two from the control group), we left the urinary catheter in place for 14 days after the procedure. Subsequent cystography did not indicate leakage and the catheter was removed (Dindo/Clavien I). One patient in the control group was conservatively treated for prolonged paralytic ileus lasting more than 5 days (Dindo/Clavien II). One patient from the treatment group experienced intestinal lesion and stercoral peritonitis because of adhesiolysis after a previously complicated appendectomy. During revision, 15 cm of ileum was resected (Dindo/Clavien IVa). Three months after the procedure, the patient was completely healed, with PSA level was < 0.008 ng/ml, and completely continent (Table 11).

## DISCUSSION

Despite all efforts, RARP carries significant blood loss in some patients. Perioperative bleeding impairs the visibility of the operating field, therefore, increases the risk of further bleeding, furthermore, worsens oncological and functional outcomes.

Radical prostatectomy is associated with a higher risk of thromboembolism. Open radical prostatectomy has a considerably higher risk of thromboembolic events (1.0–15.7%) compared to a robotic (0.2–3.7%) and laparoscopic approach (0.4–6.0%) (30). Administration of antifibrinolytics, which potentially increase the risk of thromboembolism in laparoscopic surgery for pelvic malignancy, may rise certain doubts. However, our study, in agreement with recent meta-analysis of 11 studies involving 1177 patients with malignancy, did not find increased risk of thromboembolism following treatment with tranexamic acid (26).

Despite all our efforts, we have not been able to eliminate all potential study bias. The determined level of diuresis may not reflect the reality accurately. Administration of infusions during the procedure was not standardized,

**Tab. 10** Impact on console time (minutes).

	Overall		DVC suturing		Up-grading		Up-staging		BMI
	TA	Placebo	First	Later	yes	no	yes	no	> 30
	(n = 50)	(n = 50)	(n = 38)	(n = 62)	(n = 58)	(n = 42)	(n = 17)	(n = 83)	(n = 70)
Average	132.7	130.6	135.0	129.5	133.0	129.8	124.5	133.0	128.3
SD	31.2	30.3	30.7	30.5	29.5	32.3	22.8	31.8	31.4
Q1	113.0	114.0	114.0	111.0	115.0	111.0	107.0	114.0	109.0
Q3	145.0	148.0	148.0	140.0	146.0	137.0	139.5	146.0	136.0
P-value	p = 0.754		p = 0.242		p = 0.249		p = 0.482		p = 0.008

**Tab. 11** Dindo/Clavien classification.

Grade	TA	Placebo
I	2 (prolonged lymphatic secretion, urinary leakage)	3 (prolonged lymphatic secretion, urinary leakage – 2×)
II	0	3 (brachial artery thrombosis, hematoma in the pelvis, prolonged paralytic ileus)
III	0	0
IV	2 (laparoscopic revision for haemoperitoneum, intestinal lesion)	0
V	0	0

but no statistically significant difference in the infusions between both groups was observed.

In addition, various degrees of tissue trauma were observed among the patients (e.g., adhesions of bowel, tissue fattening, prostate size, etc.) and, therefore, various lymphatic secretions were observed during and after the procedure.

We found the difference in postoperative drainage volume statistically significant, but it did not bring any clinical consequences. The drain was removed on the POD 1 or 2 with no difference between both groups.

Operations were performed by four surgeons, all of them with enough experience but with slightly different technique. Three of the surgeons performed DVC treatment after prostate removal using several turns of the barbed suture, and one surgeon after opening the endopelvic fascia with two loops of PDS stitch at the beginning of the procedure. We confirmed that ambiguous overall results were affected by mixing the two variants of the surgical procedure. There were no significant differences between both groups if DVC was treated at the end of the procedure.

Significantly lower postoperative losses in the latter case can be explained by the greater stability of the thrombus produced by the early suturing of the DVC, potentiated by the action of the tranexamic acid.

The effect of tranexamic acid on the incidence of positive surgical margins is not clear from this pilot study and will be analyzed in following study.

## CONCLUSION

Prophylactic use of tranexamic acid at the beginning of the procedure in urology, in contrast to neurosurgery, orthopaedics or cardiac surgery, is not common.

In our pilot study we demonstrated the safety of 10–20 mg/kg of tranexamic acid at the beginning of RARP. No patient from the treatment group developed a thromboembolic event within 3 months after the procedure. In addition, we showed that administration of tranexamic acid at the beginning RARP significantly reduces the decrease in haemoglobin after the procedure, when treating the DVC at the beginning of the procedure. The results will be verified in following study in a larger patient population.

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## CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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# Comparing the Efficacy of Sequential and Standard Quadruple Therapy for Eradication of *H. Pylori* Infection

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## ABSTRACT

**Background:** The aim of this study was comparison the effectiveness of sequential and standard quadruple therapy on eradication of *H. pylori* infection.

**Methods:** This clinical trial study was conducted on 160 patients with dyspepsia or gastroduodenal ulcer. Patients were randomly divided into two groups. Group A (standard regimen) received omeprazole, amoxicillin, clarithromycin and bismuth subcitrate for 2 weeks. Group B (sequential regimen) received omeprazole and amoxicillin in 5 days and omeprazole, tinidazole and levofloxacin in 5 days. After the end of treatment regimens, 20 mg omeprazole was administered twice daily for 3 weeks. *H. pylori* eradication was assessed 2 months after antibiotic treatment via fecal antigen.

**Results:** Frequency of *H. pylori* eradication in group A and B was observed in 55 (68.8%) and 63 patients (78.8%), respectively. No significant difference was seen between two groups, regarding *H. pylori* eradication ( $p = 0.15$ ). The most common side effects in group A, B were bitterness of mouth (63.8%) and nausea (16.2%), respectively ( $p < 0.001$ ).

**Conclusion:** Although no significant difference was seen between two groups regarding eradication of *H. pylori* infection, higher rate of *H. pylori* eradication was seen in group B than group A. Thus, sequential regimen was a more appropriate regimen with fewer complications.

## KEYWORDS

*H. pylori* infection; Sequential therapy; standard triple-drug therapy; eradication

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## INTRODUCTION

*Helicobacter pylori* (*H. pylori*) infection is a worldwide and chronic infection. Its incidence is related to several factors including rate of acquisition of infection with *H. pylori*, rate of loss of the infection, and long-term survival of bacteria in the gastric mucosa between infection and eradication (1). *H. pylori* infection is associated with incidence of gastrointestinal diseases such as peptic ulcer, gastric inflammation and gastric cancer (2). It may lead to dyspeptic symptoms via changing the gastric acid secretion (3, 4), post-infective altering gastroduodenal mucosa and activating inflammation of gastric mucosa (4). The prevalence of *H. pylori* infection in developing countries is greater compared to developed countries (3). Furthermore, this prevalence varies in different countries and geographic regions of Asia (5). In this regard, prevalence rate in Japan, China, and Singapore is 39%, 58%, and 31%, respectively. Moreover, report of *H. pylori* infection rate is different in various areas of Iran (6-7).

Eradicating *H. pylori* prevents the recurrence of disease, decreases the risk of gastric cancer and heals peptic ulcers (8). In addition, after treating with antibiotics, other *H. pylori*-associated disorders including chronic atrophic gastritis, intestinal metaplasia and mucosa-associated lymphoid tissue can be regressed (8). However, an important issue in treatment of anti-*H. pylori* is antibiotic resistance (9) which has an effect on treatment efficacy (10).

Several regimens have been assessed for therapy of *H. pylori* infection in clinical trial studies (11-15). Despite many studies in this regard, the optimal therapeutic regimen is still unclear. Recently, common therapies rely on combination of antimicrobial factors such as levofloxacin, metronidazole, amoxicillin and proton pump inhibitors. Clarithromycin based regimens are considered as standard triple treatment. Recently, increasing resistance to standard antibiotic therapy for *H. pylori* infection has been reported (16-23).

Some studies have shown the performance of sequential therapy for eradication of *H. pylori* infection (24-26). In addition, many studies have shown superiority of sequential therapy on standard triple and quadruple therapy (22-27).

Given that prevalence of *H. pylori* infection in Iran is high (9) and few studies have evaluated efficacy of these two treatments as the first line therapy for *H. pylori* infection in our country, the aim of current study was comparison the effectiveness of sequential therapy and standard quadruple therapy on eradication of *H. pylori* infection.

## MATERIALS AND METHODS

This clinical trial study was conducted on patients with dyspepsia or gastroduodenal ulcer referred to Shahid Beheshti hospital, Kashan, Iran during 2018. After taking consent from patients, current research was approved by Kashan University of Medical Sciences.

Inclusion and exclusion criteria were as following.

## INCLUSION CRITERIA SELECTION

- Patients over 18 years old with dyspepsia or gastroduodenal ulcer
- Confirmation of *H. pylori* infection by fecal antigen or endoscopic pathological findings
- Willingness to participate in the study

## EXCLUSION CRITERIA SELECTION

- Previous eradication of *H. pylori*
- Use of any type of antibiotics and PPI during the 4 weeks prior to the study
- Pregnant and lactating women
- Patients with renal failure
- Patients with untreated heart failure
- Patients with history of gastrectomy or complicated peptic ulcer

Then, 160 patients were selected and randomly divided into two groups ( $n = 80$ ). Group A (standard 14-day treatment regimen) received omeprazole (20 mg b.d), amoxicillin (1 gr b.d), clarithromycin (500 mg b.d) and bismuth subcitrate (240 mg b.d) for 2 weeks. Group B (sequential regimens) received omeprazole (20 mg b.d) and amoxicillin (1 gr b.d) during 5 days and omeprazole (20 mg b.d), tinidazole (500 mg b.d) and levofloxacin (500 mg b.d) during 5 days (10 days). After the end of treatment regimens, 20 mg omeprazole was administered twice daily for 3 weeks.

*Helicobacter pylori* eradication was assessed at least 2 months after the end of antibiotic treatment or at least 2 weeks after omeprazole discontinuation via fecal antigen. Information including age, sex, history of *H. pylori* infection, history of non-steroidal anti-inflammatory drugs and alcohol intake and smoking were extracted from medical records.

## STATISTICAL ANALYSIS

Data were entered SPSS, version 19. Chi square test and Fisher exact test were used for analysis of data. P-value < 0.05 was considered statistically significant.

## RESULTS

In current study, 160 patients were classified to two groups. The mean age of patients in group A and B was  $45.92 \pm 14.18$  and  $41.43 \pm 13.61$  ( $p = 0.043$ ).

Other characteristics of patients in two groups are shown in Table 1.

As shown in Table 1, no significant difference was seen between two groups, in terms of characteristics such as sex, smoking, History of *H. pylori* infection and Taking non-steroidal anti-inflammatory drugs ( $p > 0.05$ ).

Frequency of *H. pylori* eradication in two groups is shown in Table 2.

As shown in Table 2, no significant difference was seen between two groups, regarding *H. pylori* eradication ( $p > 0.05$ ).

Frequency distribution of side effects in two groups is demonstrated in Table 3.



**Tab. 1** Characteristics of patients in two groups.

Variables	Standard regimen (A)	Sequential regimen (B)	p-value Chi Square test
Sex			
Men	36 (45%)	28 (35%)	0.197
Women	44 (55%)	52 (65%)	
Smoking	11 (13.8%)	6 (7.5%)	0.200
Taking NSAIDs	18 (22.5%)	20 (25%)	0.710
History of <i>H. pylori</i> infection	5 (6.2%)	7 (8.8%)	0.548

NSAIDs: Non-steroidal anti-inflammatory drug

**Tab. 2** Frequency of *H. pylori* eradication in two groups.

Eradication	Standard regimen N (%)	Sequential regimen N (%)	p-value Chi Square test
Yes	55 (68.8)	63 (78.8)	0.151
No	25 (31.2)	17 (21.2)	

**Tab. 3** Frequency distribution of side effects in two groups.

Complications	Standard regimen	Sequential regimen	p-value Fisher exact test
Headache	0 (0)	2 (2.5)	<0.001
Dizziness	0 (0)	3 (3.8)	
Bitterness of mouth	51 (63.8)	10 (12.5)	
Nausea	1 (1.2)	13 (16.2)	
Stomach ache	0 (0)	3 (3.8)	
Diarrhea	0 (0)	2 (2.5)	
Tendonitis	0 (0)	8 (10%)	
joint pain	0 (0)	2 (2.5)	
Skin lesions	0 (0)	1 (1.2)	
No complication	28 (35)	36 (45)	

As shown in Table 3, the most common side effect in group A and B was bitterness of mouth and nausea, respectively ( $p < 0.001$ ). Moreover significant differences was observed between two groups, regarding bitterness of mouth ( $p < 0.001$ ) (Chi square test).

Logistic regression analysis of studied variables is presented in Table 4.

After eliminating confounding effect of independent variables, there was no significant difference between two groups, regarding eradication of *H. pylori* infection (Table 4).

## DISCUSSION

*H. pylori* infection is not associated with symptoms in 50% of cases. However, some individuals develop inflammation of the gastritis or ulcers in the stomach or upper

**Tab. 4** Logistic regression analysis of studied variables in treatment of *H. pylori* infection.

	B	S. E.	Wald	df	P
Group	0.5210	0.381	1.866	1	0.172
Gender	0.0370	0.421	0.008	1	0.930
Age	-0.0060	0.013	0.189	1	0.664
NSAID taking	-0.0621	0.412	2.271	1	0.132
Smoking	-0.0327	0.627			
History of <i>H. pylori</i> infection	-0.0694	0.635	1.193	1	0.275
Constant	1.2870	0.712	3.272	1	0.070

small intestine (28–31). Moreover, *H. pylori* infection causes mortality and morbidity with an economic impact, thus requiring a proper therapeutic approach. Physicians usually treat stomach pain and ulcers created by *H. pylori* via combination of various antibiotics for several days. Recently, increasing resistance to standard antibiotic therapy for *H. pylori* infection was reported (16–22). Actually after standard therapy, infection was observed in one of every six patients with peptic ulcer disease. Therefore, *H. pylori* treatment is a challenge for physicians and no current first-line therapies are capable to treat the infection in all treated individuals (32). Based on findings of recent studies, sequential therapy is identified as first-line therapy in treatment of patients with *H. pylori* infection (32).

The findings of current study showed that the sequential regimen was superior to the quadruple therapy in the treatment of *H. pylori* infection, although no statistically significant were observed between two groups. In this study 78.8% of patients in sequential group and 68.8% of patients in quadruple diet group have recovered. Vaira et al., assessed sequential therapy versus standard triple therapy for eradication of *H. pylori*. The findings showed that eradication with sequential therapy was greater than standard therapy in these patients, which was consistent with our study (32).

Sánchez-Delgado et al., assessed ten-day sequential therapy for *H. pylori* eradication. They selected 139 patients and sequential regime consisted of a 10-day treatment such as a proton pump inhibitor b.d., 1 g b.d. amoxicillin for the first 5 days, followed by a PPI b.d., 500 mg b.d. clarithromycin and 500 mg b.d. metronidazole for the next 5 days. According to findings of this study, eradication was seen in 117 out of 129 patients who returned. It seems that sequential treatment is effective for eradicating *H. pylori* (33). Zhou et al., in China assessed sequential therapy regimen compared to conventional triple therapy for *H. pylori* eradication. Then, patients in group A received clarithromycin (500 mg), esomeprazole (20 mg) for the first 5 days, following esomeprazole (20 mg), clarithromycin (500 mg), amoxicillin (1000 mg) for the remaining 5 days. Group B received esomeprazole (20 mg), amoxicillin (1000 mg) for 5 days, followed by clarithromycin (500 mg), esomeprazole (20 mg), and amoxicillin (1000 mg) the remaining 5 days. The findings of this study showed that both treatments can alleviate symptoms in patients. Moreover, they believed that sequential therapy was better than standard

triple therapy (24). Marshall et al., compared efficacy of sequential therapy with standard triple therapies during 7–10 days. The findings showed that the success of sequential regimen was higher than standard triple therapies during 7–10 days (34). Gatta et al., reported eradication rate following triple therapy and sequential therapy were 35.1% and 83.9%, respectively (35).

Varia et al., reported 7–14 days triple therapy is reducing around the world with unsatisfactory low eradication rate in various country. They believed that sequential therapy is the most effective in first-line therapy and had superiority over standard triple therapy on more than 2300 treated patients. In addition, they reported that the sequential therapy is successful against those clarithromycin-resistant strains that have the A2143G point mutation, which significantly reduces the effectiveness of standard triple therapy (32). The precise mechanism of sequential therapy was unknown. There are several reasons, but all remain unconfirmed at this time.

One of the reasons is that reducing the bacterial density in the stomach via medications including amoxicillin and improving the efficacy of subsequently administered combination such as tinidazole and clarithromycin (36).

In addition, the most common side effects in group A and B was bitterness of mouth and nausea, respectively. Moreover, significant difference was observed between two groups, regarding side effects. Kaboli et al., compared sequential regimen and standard therapy for *H. pylori* eradication. The findings showed significant difference between two groups, regarding side effects, which was consistent with our study (37). Aminian et al., compared sequential regimen and standard quadruple therapy in patients with dyspepsia. The findings showed that there was significant difference between sequential regimen and standard quadruple regimen, considering side effects (38). This study also was consistent with our study.

It is noteworthy that the eradication rate of *Helicobacter pylori* in the two treatment groups was compared with controlling confounding variables of age, sex, use of non-steroidal anti-inflammatory drugs, smoking and family history of *Helicobacter pylori* infection via logistic regression analysis (Table 4). This caused to control the effect of confounding factors.

## CONCLUSION

Although no significant difference was seen between two groups in terms of eradication of *H. pylori* infection, higher rate of eradication of *H. pylori* infection was observed in sequential treatment regimens than standard regimens. Therefore, it was considered as a more appropriate treatment regimen compared to standard regimens in first-line therapy of *H. pylori* infection. In addition, this medication regimen was associated with fewer side effects.

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# Evaluation of Midpalatal Suture Ossification Using Cone-Beam Computed Tomography: A Digital Radiographic Study

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## ABSTRACT

**Background:** Cone beam computed tomography (CBCT) imaging techniques are the recent rage in the field of oral diagnostic imaging modality. It is noninvasive, faster and lacks anatomic superimposition. Earlier maxillary occlusal radiographs were used to assess and evaluate the mid palatal suture, but being a two dimensional imaging modality it could not assess the ossification process which takes place in multiple planes mostly due to curved nature of the palate. In this study we assessed the mid palatal suture morphology and classify them according to the variants using CBCT images.

**Materials and methods:** A total of 200 CBCT scans (95 males and 105 females) were evaluated in the present study from the archives of an imaging center. As per Angelieri classification the midpalatal suture was classified into five categories (A–E) depending on the degree of ossification that had taken place. Statistical analysis was done by Chi Square test using SPSS version 23.0.

**Results:** There is statistically significant difference present in the stages of maturity of mid palatal suture in various age groups with Stage B is most common in Group 1 (50%), Stage C most common in Group 2 (60%) and Group 3 (40%) and Stage E more common in Group 4 (50%).

**Conclusion:** The results of the present study showed a wide variation in the initiation time and the degree of ossification and morphology of the midpalatal suture in different age groups. Although there was an increase in the closure of the suture with aging, age is not a reliable criterion for determining the open or closed nature of the suture. This finding is important in providing an idea as to how diverse is the ossification of maxillary sutures.

## KEYWORDS

CBCT; midpalatal suture; ossification; maturation; maxillary suture

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**INTRODUCTION**

A pristine and aesthetically beautiful smile is important to many, and so to get their smiles as beautiful as possible more and more people are opting for orthodontic corrections. Moreover, it is not just for aesthetics that orthodontic treatments are done, the more the awareness penetrates deep into the society, the more people understand that proper alignment of teeth not only helps in aesthetics but also helps in optimum function and boosts confidence and improves the quality of life of a person. For proper orthodontic planning, CBCT plays a significant role. CBCT is the recent rage in craniofacial imaging modality which provides for accurate three-dimensional images without any overlapping.

Unlike other cranial sutures in our body, the mid palatal suture is the solitary suture that might not close up in the elderly. This midpalatal suture plays a vital role in a certain orthodontic procedure like Rapid Maxillary Expansion (RME) which helps in correcting transverse discrepancies of the maxilla by applying force with in-office appliances to expand the maxillary width (1, 2). However, with the fusion of the maxillary suture, the procedure of correcting the transverse discrepancy cannot be done only with appliances and needs to be surgically assisted thus giving rise to a procedure known as Surgically Assisted Rapid Maxillary Expansion (SARME) (3). Several studies on the morphology, initiation time and degree of fusion of the suture in different individuals have yielded wide variations concerning initiation time and progression of suture closure; a few cases over 18 years of age have reported completely open sutures, and hence the decision whether to do an RME or SARME cannot be based only on the chronological age and needs to be assessed as per individual patients (4, 5).

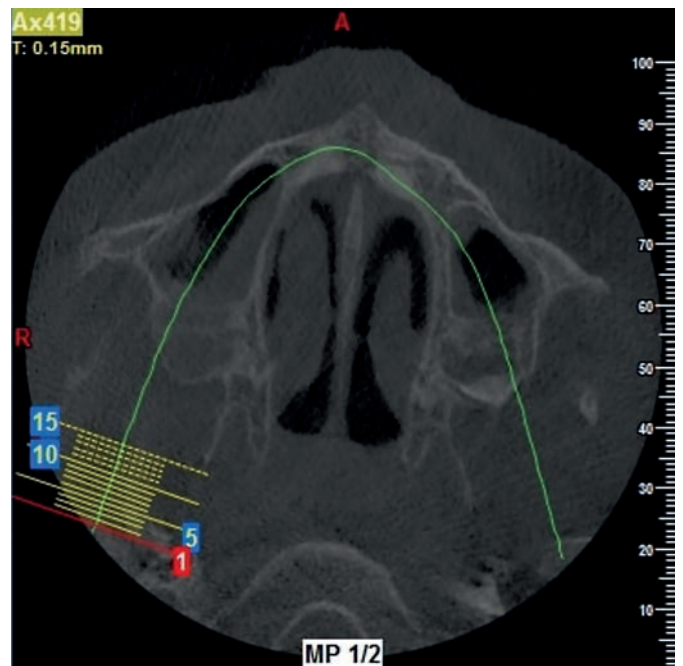
The use of maxillary occlusal radiographs did earlier the assessment of the midpalatal suture, but this method was not without flaws. Since the occlusal radiographs are a 2D representation of a 3D structure and so due to the superimposition of the nasal structures and the vomer bone on the midpalatal area, misinterpretation of the radiograph and discrepancy with morphologic findings are possible. This is where the CBCT comes in as the latest offering in the field of dental imagery solving the problems faced with the 2D imaging techniques. CBCT provides high-resolution 3D images of craniofacial structures (6, 7). Added advantages are high dimensional accuracy, noninvasive nature, time-saving, easy access, and lack of any anatomic superimpositions. In light of the above literature, given the discrepancies amongst the results of studies regarding the initiation time and maturation of the midpalatal suture (8-10), this study was done to evaluate the midpalatal suture morphology in various age groups.

**MATERIALS AND METHODS**

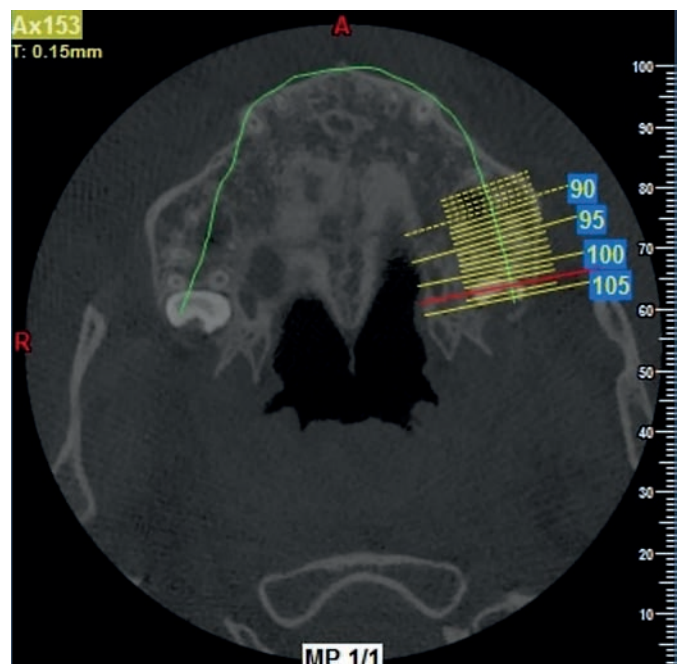
CBCT is easily accessible these days’ orthodontists are not leaving anything to chance and are referring for CBCT scans when in doubt and are asking for an opinion regarding the radio analysis of mid palatal suture ossification

and maturation. The sample of this particular study has been taken from one such scan center where patients are referred for CBCT analysis.

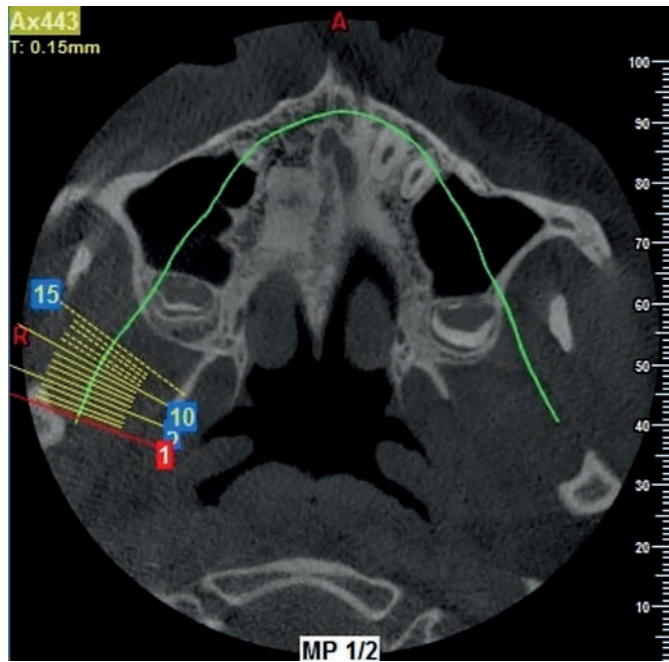
A total of 200 full volume CBCT scans showing the entire maxilla using the NewTom®Giano CBCT machine with the NNT software were collected from the archival records of the CBCT scan centre. The ethical clearance to conduct the study was obtained from the Institutional Ethical Committee. Care was taken not to include any scans of subjects who were undergoing orthodontic treatments or those having any pathologies in the maxilla. Out of the 200 scans, 95 were of male and 105 were of female



**Fig. 1** Stage A, the mid palatal suture is almost a straight high-density sutural line with no or little inter digitations.



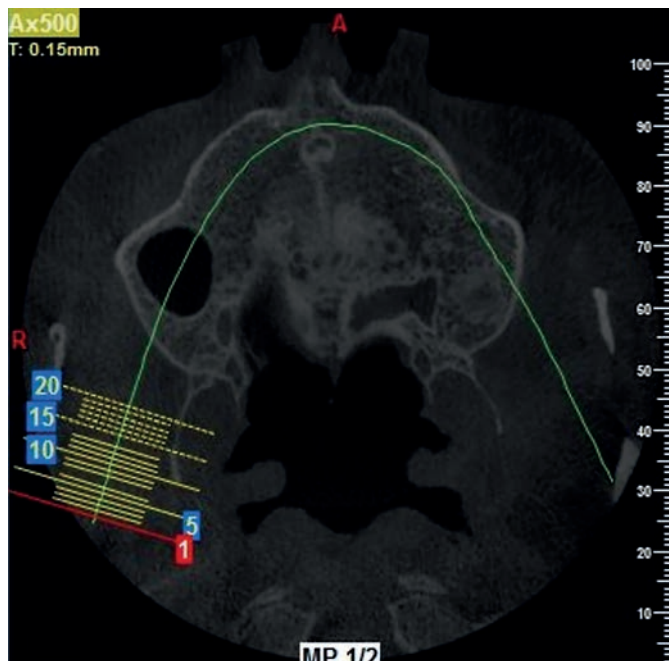
**Fig. 2** Stage B, the mid palatal suture assumes an irregular shape and appears as a scalloped high density line.



**Fig. 3** Stage C, the mid palatal suture appears as 2 parallel, scalloped, high-density lines that are close to each other, separated by small low-density spaces in the maxillary and palatine bones.



**Fig. 5** Stage E, fusion of the mid palatal suture has occurred in the maxilla. The actual suture is not visible in at least a portion of the maxilla.



**Fig. 4** Stage D, the fusion of the mid palatal suture has occurred in the palatine bone, with maturation progressing from posterior to anterior. In the palatine bone, the mid palatal suture cannot be visualized at this stage.

patients with age ranging from 11 years to 50 years. The precaution was taken to not in any way disclose the identity of the scans. The scans were obtained through a period of 12 months from November 2018 to October 2019. The scans were then grouped into 10-year age groups, like 11–20years, 21–30years, 31–40years, and 41–50years. These groups were named as group 1, group 2, group 3 and group 4, respectively.

The classification of the morphology of the midpalatal suture was done based on the classification done by Angileri et al. in their study (9). The classification is as follows:

In stage A, the mid palatal suture is almost a straight high-density sutural line with no or little interdigitations (Fig. 1).

In stage B, the midpalatal suture assumes an irregular shape and appears as a scalloped high-density line (Fig. 2).

In stage C, the midpalatal suture appears as 2 parallel, scalloped, high-density lines that are close to each other, separated by small low-density spaces in the maxillary and palatine bones (between the incisive foramen and the Palatino-maxillary suture and posterior to the Palatino-maxillary suture) (Fig. 3).

In stage D, the fusion of the midpalatal suture has occurred in the palatine bone, with maturation progressing from posterior to anterior. In the palatine bone, the midpalatal suture cannot be visualized at this stage, and the para-sutural bone density is increased (high-density bone) compared with the density of the maxillary para-sutural bone. In the maxillary portion of the suture, fusion has not yet occurred, and the suture still can be seen as 2 high-density lines separated by small low-density spaces (Fig. 4).

In stage E, the fusion of the midpalatal suture has occurred in the maxilla. The actual suture is not visible in at least a portion of the maxilla. The bone density is the same as in other regions of the palate (Fig. 5).

The scans were assessed in a well-lit room by a single observer, twice after a gap of 5 days to do away with any intra-observer ambiguity. A weighted kappa coefficient was calculated to evaluate the intraexaminer agreement for the classification of the midpalatal suture maturation stages. The results of the intraclass correlation coefficient (ICC) test revealed high reliability between the two

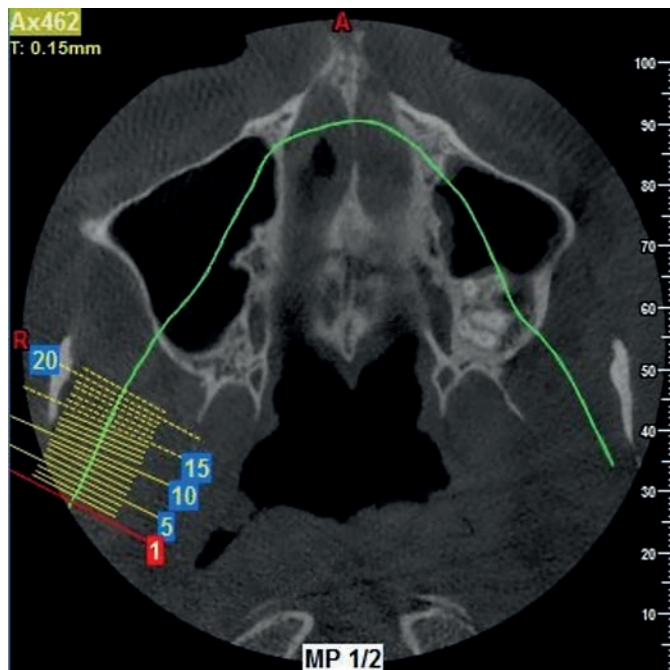


Fig. 6 Stage D', modification of Stage D.

assessments for all regions (ICC > 0.8). Thus the scans were classified into Stages A, B, C, D or E and were grouped as per the age groups. It was then analyzed by the Chi-Square test to find out if any significant differences are there amongst the groups. The statistical analysis was done using SPSS version 23.0, Chicago, USA.

**RESULTS**

The observer analyzed the 200 scans, they were classified under the 4 age groups, which were the groups 1-4, and the scans were distributed among the age groups as per the Angelieri classification. After analyzing twice by the observer, there were no statistically significant changes in both the readings.

A total of 15 scans were in stage A, 40 were in stage B, 70 scans were classified as stage C, 25 were in stage D and 40 in stage E. Since A, B and C were the groups in which there is no fusion of the mid palatal suture it can be said that over 50% of the samples had no fusion in the midpalatal suture.

Apart from the 5 morphological classifications mentioned by Angelieri et al. (9), we found that there was one more variant of midpalatal suture maturation where the ossification process was not continuous but in bouts along the suture line. We named this as stage D' which was a modification of D, and 10 samples were there in this class (Fig. 6). A noteworthy thing in the results is that about 75 scans of subjects over 20 years of age were either in class A, B or C. that means 50% of the samples over 20 years of age had open sutures (Table 1 and 2).

There was a statistically significant difference in the stages of maturity of the mid palatal suture in various age groups with Stage B (50%) is most common in Group 1, stage C most common in Group 2 (60%) and Group 3 (40%) and Stage E more common in Group 4 (50%).

The results showed a gradual maturation in ossification of the midpalatal suture with an increase in age, but a specific age could not be determined in which the suture fuses completely. Furthermore, no significant finding could be assessed regarding the maturation pace concerning gender.

**DISCUSSION**

The increasing awareness of oral health and smile appeal is making people more concerned with proper aesthetics and overall health of the oral cavity. This is one of the main reasons for the ever-increasing cases at the orthodontists. The dental treatment being individual-specific as always, the midpalatal suture ossification of an individual carries an important role in orthodontic treatment if there is a discrepancy in the palatal width. Since after many previous studies, no particular age could be confirmed for the fusion of the midpalatal suture this particular study was

Tab. 1 Mean and SD of age in various age groups according to gender.

Age Group	Gender	N	Minimum	Maximum	Mean	Std. Deviation
11-20 years	Female	35	11.0	17.0	14.71	1.98
	Male	15	13.0	18.0	15.33	2.52
	Total	50	11.0	18.0	14.90	2.02
21-30 years	Female	20	21.0	30.0	23.50	4.36
	Male	30	22.0	30.0	25.67	2.88
	Total	50	21.0	30.0	24.80	3.49
31-40 years	Female	25	31.0	40.0	35.40	4.28
	Male	25	31.0	40.0	36.00	4.58
	Total	50	31.0	40.0	35.70	4.19
41-50 years	Female	25	45.0	50.0	46.80	2.17
	Male	25	41.0	50.0	45.20	3.83
	Total	50	41.0	50.0	46.00	3.06

**Tab. 2** Comparison of stages of mid palatal suture maturation.

Stage	Group				Total
	1	2	3	4	
A	15 (30%)	0	0	0	5 (7.5%)
B	25 (50%)	5 (10%)	5 (10%)	5 (10%)	40 (20%)
C	10 (20%)	30 (60%)	20 (40%)	10 (20%)	70 (35%)
D	0	5 (10%)	15 (30%)	5 (10%)	25 (12.5%)
E	0	5 (10%)	10 (20%)	25 (50%)	40 (20%)
X [D']	0	5 (10%)	0	5 (10%)	10 (5%)
Total	50 (100%)	50 (100%)	50 (100%)	50 (100%)	200 (100%)
Chi Sq	154.714		P value	0.000*	

\* Statistically significant ( $p < 0.01$ )

done to assess the ossification process on CBCT scans and classify as per their maturation progress. CBCT was chosen as it is better for any 2D imaging modality for its 3D analysis and majorly due to no structural overlap.

The results of the present study with the degree of ossification of the suture in the age groups 1–4, showed that ossification increased with ageing; however, some cases of completely open sutures were observed in people over 20-year age groups.

A study done on patients over the age of 70 years revealed that the midpalatal suture is the only suture that might not close completely even in the elderly (11). Further, in another study subjects more than 40 years of age, showed a few cases of incomplete ossification of the suture, which was similar to the findings in our study (4). In one more study with the use of CBCT images, in only 13% of the adult subjects completely closed sutures were detected (12).

There was no significant relationship between the closure of the suture and age drawn from the results of 30 micro-CT analyses in a study carried on 28 patients, 14 to 17 years of age (8). Based on a hypothesis by Cohen MM, there is no relationship between the termination of growth and closure of the suture, i.e., even if 95% of the growth of the maxilla ends at 7 years of age, the suture is not necessarily closed (13).

The decrease in functional forces of muscles with ageing, teeth loss and soft diet, contribute to the open suture in adults (14); thus, the relative course of development such as morphology and ossification process of the midpalatal suture contrary to other cranial sutures depends on the masticatory forces exerted on the maxillary bone during life.

In a study by Angelieri et al. on CBCT images of 140 patients, subjects over 11 years of age exhibited all the developmental stages for the midpalatal suture. The chronological age was an invalid criterion for determining the developmental stage of the midpalatal suture during growth (9). In the present study, too, evaluation of the morphology of the suture in 10-year age groups revealed wide variations in subjects over 20 years of age, with 50% of the subjects over 20 years of age in stages A, B, and C of the development of the suture; in these stages, completely open sutures are observed.

In a study by Fricke-Zechet et al. it was reported that different individuals have varied morphologic changes in suture which are not completely related to age (15). In the present study, the suture morphology did not reveal any significant differences between genders, which are consistent with the results of a study by Revelo et al. (16). The results of the present study showed the posteroanterior direction of the ossification of the suture. The results of the present study showed the posteroanterior direction of ossification of the suture, similar to the study done by Melsen et al. (17). However, with exceptions where there were interrupted bouts of ossification along the suture line which could be said as a modification of D. Even though there was an increase in the closure of the suture with ageing, nevertheless, age is not a consistent measure for determining the open or closed nature of the suture.

## CONCLUSION

There is ample variation in the initiation time and the degree of ossification and morphology of the midpalatal suture in different age groups. Even though there was an increase in the closure of the suture with ageing, stillage is not a consistent criterion for determining the open or closed nature of the suture. This finding is important in preparing an appropriate orthodontic treatment plan, irrespective of the patient's age. Further, studies with a larger sample size will spread more light on the maturation process of midpalatal suture.

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# Massive Traumatic Subcutaneous Emphysema

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Diana Fernandes\*, Sara Pereira, Celeste Guedes, David Silva

## ABSTRACT

74 year-old-man, former smoker, with chronic obstructive pulmonary disease GOLD grade 4, group D, with emphysema component, treated in a pulmonary rehabilitation program, on oxygen therapy and nocturnal bi-level positive airway pressure (BiPAP) ventilation. During the night he had a traumatic rib fracture (5–11th right ribs) but still he used BiPAP ventilation during the sleep. In the morning after he presented with a diffuse and massive emphysema in the face, thorax and abdominal regions. On physical examination, the patient presented with massive swelling and crepitus on palpation. A chest computed tomography (CT) scan confirmed a diffuse subcutaneous emphysema and revealed a mediastinal emphysema and bilateral small pneumothorax. A fast resolution of the emphysema was of paramount importance as the patient was severely agitated due to his inability to open both eyes, and the need to reintroduce BiPAP ventilation as soon as possible. It was placed a fenestrated subcutaneous catheter on left hemithorax and a subcutaneous ostomy on right hemithorax for comparative purpose. It was also performed a confluent centripetal massage towards drainage orifices, with immediate and substantial improvement of emphysema, especially in left hemithorax, and progressive ocular opening. Further emphysema absorption occurred during hospitalization.

## KEYWORDS

chronic obstructive pulmonary disease; extensive subcutaneous emphysema; venous catheter; pneumomediastinum; subcutaneous emphysema

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## INTRODUCTION

Subcutaneous emphysema is often nothing more serious than a cosmetic problem (1, 2). Although it can be extremely uncomfortable for the patient, even when it is severe, subcutaneous emphysema rarely has pathophysiologic consequences (1, 2). The widely employed methods of therapy, which include placing chest tubes or lacerating the skin on the anterior chest, are time-consuming and uncomfortable (1, 2).

Here, we report a case of extensive subcutaneous emphysema following trauma, treated with an easily constructed fenestrated venous catheter.

## CASE REPORT

We report a case of a 74-year-old man admitted to the hospital with extensive subcutaneous emphysema. He had a history of chronic obstructive pulmonary disease GOLD grade 4, group D, with emphysema component, treated in a pulmonary rehabilitation program, on oxygen therapy and nocturnal bi-level positive airway pressure (BiPAP) ventilation. Other past medical history included arterial hypertension, dyslipidemia, epilepsy and was a former smoker.

He had a traumatic rib fracture (5–11th right ribs) during the night after falling from his own height and hitting the ground with his right hemithorax region. After that he went to sleep maintaining BiPAP ventilation until morning. In the morning after, he was admitted at emergency room with a diffuse and massive emphysema on his face, thorax and abdominal regions.

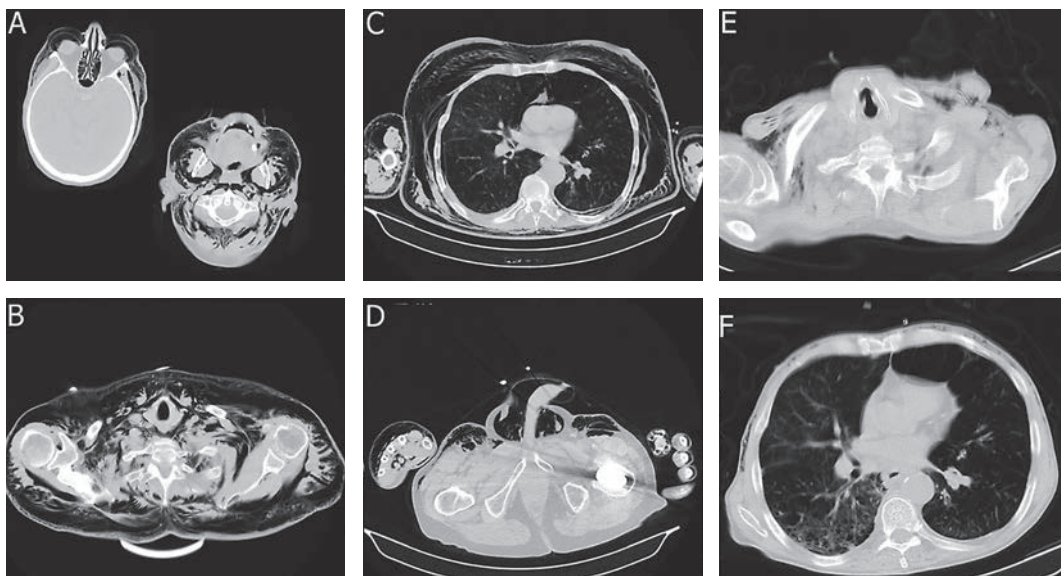
On physical examination, the patient presented a massive swelling and crepitation on palpation. His blood pressure was 107/83 mmHg, pulse rate of 100/min and regular, SpO<sub>2</sub> 100% with FiO<sub>2</sub> 100% and axillary temperature of 36 °C. Pulmonary and cardiac auscultation were difficult to assess due to massive emphysema.

Arterial blood gas analysis revealed type II respiratory failure; and electrocardiogram showed sinus rhythm, with global low voltage. Complete blood count, liver and renal function tests were normal. Chest computed tomography scan confirmed a diffuse subcutaneous emphysema and showed mediastinal emphysema and bilateral small pneumothorax (Figure 1).

He was transferred to the intermediate care unit for clinical surveillance and close monitoring.

A fast resolution of the emphysema was required as the patient was severely agitated due to bilateral ocular opening impairment and the need to reintroduce BiPAP ventilation as soon as possible. Hence, under local anesthesia and antiseptic measures, a Redon catheter (12 French, B.Braun) was inserted on left hemithorax, insertion point located 4th next to left side of sternum. The catheter was placed using the Seldinger's technique: the subcutaneous left hemithorax was punctured through a blunt dissection with a sharp hollow needle; a guidewire is then advanced through the lumen of the needle, and the needle is withdrawn; finally the catheter was insert and the guidewire was withdrawn. Then, it was fixed to the skin with 2/0 silk and adapted to drain bags. On the right hemithorax, a subcutaneous ostomy with about 1cm, was performed for comparative purpose (Figure 2).

Afterwards, it was performed a compressive massage in a centripetal fashion, towards drainage orifices, with immediate improvement of emphysema. This had led to a significant improvement with progressive ocular opening over the next hour. Conservative treatment with oxygen and inhalers were continued. The swelling decreased substantially and BiPAP ventilation was discontinued on the basis of the results of arterial blood gas analysis and following case discussion with pulmonology team. During the stay at intermediated care unit, the patient developed a nosocomial pneumonia managed with empirical antibiotic therapy – piperacillin-tazobactam. No other major inter-currence was described.



**Fig. 1** Computed tomography scans showing diffuse subcutaneous emphysema, mediastinal emphysema and bilateral small pneumothorax.



**Fig. 2** In figure A the patient shows an exuberant emphysema. In figure B the fenestrated subcutaneous venous catheter (12 French) was inserted on left hemithorax, and subcutaneous ostomy, with about 1cm on right hemithorax was performed. In figure B the patient shows a important decrease of emphysema one day after the procedures.

Patient was transferred from intermediated care unit to medical ward at day 5, after further emphysema absorption.

The subcutaneous venous catheter was removed without any signs of inflammatory reaction 9 days later. The treatment for nosocomial pneumonia was completed. The patient was discharged home with complete symptoms resolution.

## DISCUSSION

Aberrant extra-alveolar air starts with alveolar rupture and leakage of air into the pulmonary interstitium (3–5). This air subsequently tracks along the perivascular space to the mediastinum, a phenomenon known as the Macklin effect (3–5). Further extension along tissue planes leads to subcutaneous emphysema, pneumopericardium and pneumoperitoneum (3–5). This pathophysiologic process, first described by Macklin in 1939, is summed up in three steps: alveolar ruptures, air dissection along bronchovascular sheaths, and spreading of this pulmonary interstitial emphysema into the mediastinum (3–5). When leakage of air is greater than reabsorption, progressive accumulation in various tissue planes occurs (3–5). Commonly, the least resistance to expansion is offered by subcutaneous tissue, leading to worsening subcutaneous emphysema (SE) (3–5).

Subcutaneous emphysema often presents a therapeutic dilemma (1, 2). Patients with severe SE may develop dysphagia or vision problems because of periorbital swelling, just like in this case (1, 2). More severe complications have been rarely reported, which include respiratory failure, pacemaker malfunction, airway compromise, and tension phenomena (1, 2).

A number of techniques have been employed to treat subcutaneous emphysema (2, 6–10). These include infraclavicular incisions, placement of additional chest tubes either in the intrapleural space or subcutaneously, tracheostomy, and large-bore subcutaneous drains with or

without suction (2, 6–10). In this patient two techniques were used: on the right side an infraclavicular incision was performed and on the left side a subcutaneous catheter was placed. The latter equipment is widely available and easily modified, minimally invasive, it is simple to insert, maintain and is effective, painless, does not require suction, and is less likely to produce a scar (2, 6–10). We have not found any significant difference between the two techniques used in this patient, but we consider infraclavicular incision to be a best suited technique due to its simplicity and associated with a lesser risk of infection.

A key step in this process is to increase the interstitial hydrostatic pressure by sequential massage from the face downwards and arm upwards towards the catheter, that should be done 3 to 4 times per day (6). The resolution of emphysema after the catheter placement started only after the above step (6).

To confirm the adequacy of the compressive massage, Srinivas R et al. suggested the placement of an underwater trap and visualization of bubbling as endpoints for adequate compressive massage (6).

Suri JC, Ray A et al., reported in a case report that resolution of swelling was noticed after 8 hours and the catheter was removed after 24 hours (2). According to these authors, this technique ensured an earlier resolution compared to previous reports (where the median time of improvement was 3.7 days) (2). This can be attributed to clear identification of goal to be attained by compressive massages or the sealing of air-leak concurrent to catheter insertion. Other ancillary adjuncts like adequate analgesia, cough suppression and supplemental oxygen therapy may have hastened recovery (2). In the timing of resolution, we should take in account the presence of other mechanism that could perpetuate the emphysema, as for example an ongoing leak of air or significant lung collapse. In these cases, the closure of a fistula or treatment of pneumothorax with thoracic drains should be also applied. Although no studies have yet documented for how long the catheter works, Leo F et al. defends no longer than 3 days (9). Usually, at the time of removal, the catheter is obstructed by clots and dislodged by the movements of the subcutaneous and muscular planes (9).

In the present case a significant reduction of emphysema was noted after the first massage, so we believe that the first massage done with repetitive and vigorous movements is essential for the recovery.

Although we have not experienced any problems with the catheter described here, there are two potential problems (1–2). The first one is infection but, as long as a rigorous asepsis is maintained during placement of the catheter, it is not higher than the risk of infection for central venous catheters (9); and the second is that the catheter may become blocked with blood (1–2).

In conclusion, extensive subcutaneous emphysema can be associated with extreme discomfort, anxiety, longer hospital stays and respiratory failure. Fenestrated catheters are simple to insert and maintain, and the procedure is effective, painless, minimally invasive and infrequently complicated by infection. We did not find any difference between this technique and infraclavicular incision, but we defend the use of infraclavicular incision because it is

easier to perform, less expensive, and associated with a lower number of infections. The key step is to increase the interstitial hydrostatic pressure by sequential massage.

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# Atypical Presentation of Pseudoxanthoma Elasticum in Two Siblings from North India

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Sunayana Misra\*, Ravindra Kumar Saran

## ABSTRACT

Pseudoxanthoma elasticum (PXE) is a rare hereditary disorder occurring due to metabolic defect in the liver and manifesting predominantly in the skin, eyes and arteries. It shows characteristic yellowish papules on the skin around the nape of neck along with looseness of skin over flexural surfaces. PXE shows marked phenotypic heterogeneity. Complications related to arterial wall and retinal Bruchs' membrane calcification occur later in life; early diagnosis therefore helps keep patient on follow up for development of the same. In Indian patients, classic skin changes may be missed clinically making histopathology pivotal in diagnosis and patient management.

## KEYWORDS

pseudoxanthoma elasticum; hereditary; metabolic defect; ectopic calcification

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**INTRODUCTION**

Pseudoxanthoma elasticum (PXE) is a rare multisystem disorder characterized by progressive calcification and fragmentation of elastic fibers affecting skin, cardiovascular system and retina (1). Its prevalence is estimated to be 1 in 25,000 to 100,000 individuals with slight female preponderance (1). PXE has a highly variable phenotypic spectrum with no particular ethnic or racial predilection (2).

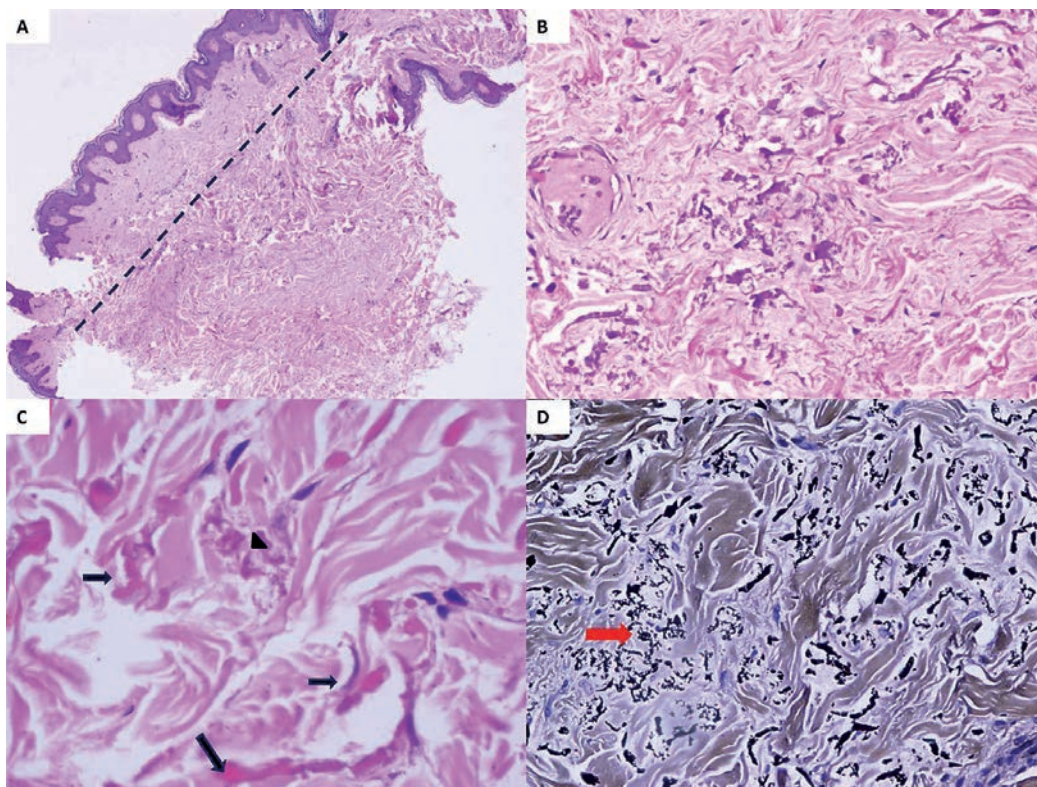
The term PXE was coined by Darrier in 1896 as the characteristic yellowish papular skin lesions give a “plucked chicken” appearance resembling xanthomas seen in hyperlipidemic disorders (3). Mutations in the ATP binding cassette C member 6 (ABCC6) gene are implicated in its etiology (4). We report this rare disorder in two siblings without any systemic complications, thus highlighting that a correct diagnosis in earlier stages of disease allows for timely follow-up of disabling complications.

**PRESENTATION OF CASES**

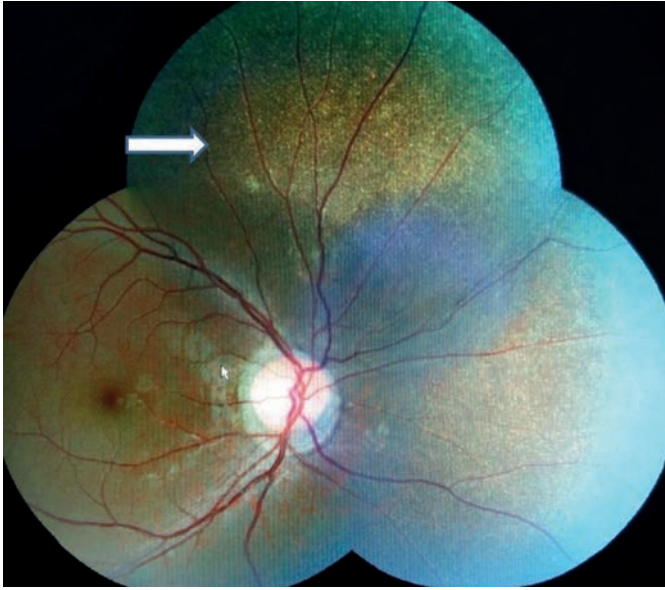
A 26-year-old Indian female (case 1) and her 18-year-old younger brother (case 2) (of non-consanguineous parentage) presented with loose skin folds over the anterior abdominal wall and axillary skin since one year which was causing aesthetic concerns (Figure 1). On general physical examination, hair, nails and mucous membranes were normal; no joint hypermobility was appreciated in



**Fig. 1** Loose skin over flexural folds (elbow and axilla) and anterior abdominal wall in case 1.



**Fig. 2** A: Skin biopsy from anterior abdominal wall shows relatively unremarkable epidermis and superficial dermis while deeper dermis shows irregular, haphazard arrangement of elastic fibers (demarcated by dash line; Haematoxylin and Eosin [HE] stain, 40×), B: Fragmentation of elastic fibers with presence of basophilic deposits on these fibers (HE, 200×), C: eosinophilic, swollen and clumped elastic fibers (arrows) with basophilic granular deposits (arrow head) (HE, 400×), D: Calcium identified as black deposits on the elastic fibers (arrow) (Von Kossa stain, 200×).



**Fig. 3** Funduscopy of case 2 revealed early changes of PXE as pigmentation around optic disc or the peau d' orange appearance of macula (arrow).

either sibling. The peripheral arterial pulses were palpable; though cord like to feel. Cutis laxa and Ehler's Danlos syndrome were kept as the clinical differential diagnoses. Other 3 siblings were unaffected at present.

Skin biopsies were performed from the affected skin over anterior abdominal wall in both cases. They showed similar morphology, though much more marked in case 1 (Figure 2A-D). Epidermis and superficial dermis were unremarkable. The reticular dermis showed fragmented elastic fibers with frayed ends which were swollen, clumped and deeply eosinophilic. Additionally, fine basophilic granular deposits were seen around the fragmented fibers which were strongly positive for Von-Kossa stain indicating them to be calcium phosphate deposits. A diagnosis of PXE was made in both cases, based on characteristic histopathology.

Based on histopathology diagnosis the cases were reviewed clinically, however classic yellowish papules were not appreciated in either sibling. Following the histopathology diagnosis, complete clinical and biochemical workup for PXE was performed. Serum lipid profile, calcium levels and hemogram were found to be within normal limits in both cases. Two-dimensional echocardiography showed mild left ventricular hypertrophy in case 1. Ejection fraction was within normal limits in both cases; no valvular or arterial calcification was appreciated. Ophthalmic examination revealed "peau d'orange" changes in the macula in case 2 (Figure 3); no ocular abnormality was noted in case 1. Both patients are on routine follow up for disease progression.

## DISCUSSION

PXE is inherited in an AR manner, although autosomal dominant (AD) and sporadic forms also occur. Over 350 mutations have been reported in the implicated *ABCC6*

(located on chromosome 16p13.1.2) gene which encodes an efflux transporter primarily expressed in liver, kidney and intestines (6). It is expressed at very low levels in the tissues directly affected by PXE. Based on these lines of evidence, PXE is thought to be a metabolic disorder with primary molecular defect in the liver<sup>5</sup> and manifestations elsewhere (skin, eyes and blood vessels).

Under physiologic conditions, *ABCC6* protein is expressed at high levels on the baso-lateral surface of liver where it facilitates the transport of anti-mineralization factors from hepatocytes to the circulation. These factors such as Feutin-A prevent precipitation of calcium/phosphate complexes in peripheral tissues. In the absence of *ABCC6* transporter activity in liver, the concentration of anti-mineralization factors in circulation and peripheral tissues is reduced, allowing mineralization of connective tissues to ensue (5). Additionally, fibroblasts in dermis, retinal Bruch's membrane and blood vessels show enhanced degradation due to elevated levels of matrix metalloproteinases (MMP) (4).

Clinical manifestations occur mainly in the skin, eyes, gastrointestinal (GI) tract and blood vessels (1). Although fully penetrant, clinical findings of PXE are rarely present at birth. Skin changes are the most frequent and also the first to appear; usually manifesting by the second or third decade of life (5, 6); they are characterized by yellowish asymptomatic papules of 1-3 mm in diameter, symmetrically distributed in the neck and flexural areas, especially the axillae with marked loosening of the skin folds. In our cases, classic skin lesions were not identified; only looseness of skin folds was observed leading to other clinical differential diagnoses. Ocular manifestations occur in the form of angioid streaks which represent breaks in the retinal Bruch's membrane due to calcium deposits (7). This may ultimately lead to the rupture of retinal vessels, with subsequent neovascularization, retinal scarring and loss of central vision. Early signs of ophthalmic involvement are seen in the form of "peau d'orange" appearance of the macula on funduscopy (7) (seen in case 2).

Calcification of medium sized arterial wall occurs, predisposing to atheromatosis. When arterial involvement is present, there are an increased propensity to GI hemorrhages, hypertension, acute myocardial infarction, cerebrovascular accident and peripheral arterial occlusion (1, 6). As cardiovascular manifestations are the last to develop, previously diagnosed cases should be on follow up long time period. Renal involvement in PXE has been reported previously (8). Thus, nephrocalcinosis and nephrolithiasis have been found in few instances as the presenting complaint in PXE (9). Of interest, some approaches in the treatment of vascular calcification in renal affected patients may represent a novel therapy in the treatment of the vascular compromise in patients with PXE (10).

Histology of PXE is characteristic. Clumped, fragmented elastic fibers and calcium deposits are found in mid and deep reticular dermis in skin. Similar changes occur in elastic fibers of the blood vessels, Bruch's membrane of the eye, endocardium and other organs. Other dermatological and systemic diseases may have PXE like skin manifestations, clinically and histologically (11) (table 1). The most important clue for diagnosis of PXE is presence of calcium



deposits in fragmented elastic fibers in the deeper layers of dermis. These histological findings should prompt a thorough cardiovascular and ophthalmic examination.

## CONCLUSIONS

PXE presents with considerable intra- and inter-familial heterogeneity; varied manifestations maybe seen within same family. Early diagnosis is crucial as it allows for follow-up for subsequent complications. In our cases, classic skin papules were not appreciated leading to clinical misdiagnosis. Hence, histopathology was pivotal in providing correct definitive diagnosis.

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# The Biometeorology of COVID-19: A Novel Therapeutic Strategy?

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## KEYWORDS

COVID 19; SARS-COV-2; climate; coronavirus

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## BRIEF COMMUNICATION

In December 2019 a novel coronavirus, SARS-CoV-2, genetically related to SARS-CoV, was first reported in Wuhan, China. The rapid spread of the virus, that has affected more and more countries, and its substantial morbidity and mortality has prompted the World Health Organization to declare the coronavirus disease (COVID 19) outbreak a global pandemic (1).

Following the spread of the virus, based on the median daily reproduction number ( $R_t$ ), it was immediately clear, as confirmed by recent biometeorological studies, that Coronavirus was initially spreading mainly in the corridor between 30° and 50° of latitude, China, Japan, Southern Korea, Iran, Northern Italy, characterized by a temperate climate. Furthermore, some countries very close to China such as Vietnam, Cambodia or Thailand were not suffering from the infection as massively as in other countries, for example the Northern Italy. Likely, in the initial phase of the spread the most affected areas of Australia and Africa were precisely those with a temperate climate such as the new west south and the city of Victoria and the South Africa (1). Interestingly, climatic factors as latitude, temperature, and humidity seem to strongly influence the propagation of the COVID 19 (2, 3).

To better understand epidemiological and spread characteristics of SARS-COV-2, the best model currently available is the human influenza virus. Human influenza incidence is characterized by a strong seasonal cycle in temperate regions depending on environmental factors that may play a role in the transmission process. For instance, previous literature data have examined temperature and humidity as factors associated with influenza propagation (4, 5).

According to Wang et al. at low temperatures the influenza virus is more stable and the respiratory droplets, which represent the containers of the viruses, persist suspended in the dry air for longer (3). In addition, the dry weather could weaken the immunity of the host and make them more vulnerable to the virus (1, 3). Again, some epidemiological studies have demonstrated that low levels of specific humidity are associated with the onset of pandemic and epidemic influenza in the North America. As a consequence, high temperature and high humidity may reduce the transmission of influenza (4). Hence, survival and transmission of influenza viruses are favored by low specific humidity conditions in temperate regions, thus causing annual winter epidemics. However, this relationship is inconsistent in tropical and subtropical regions where epidemics often occur during the rainy season and are transmitted all year round without a well-defined season. This could be due to the strong variations in temperature and humidity between day and night, typical of tropical climates, which make the climatic conditions unstable (4, 5).

All these mechanisms are also likely to apply to COVID-19 transmission. SARS-COV-2, as well as influenza virus and other viruses, seems to use humans to survive and, above all it uses humans as vectors to reach regions with more favorable climate. Actually, following the number of contagions over time, epidemiologists have observed that the SARS-COV-2 moved from East to West in

search of conditions of humidity, temperature and atmospheric pressure suitable for its survival. Indeed, SARS-COV-2 would survive better in a climate between 5 and 11 degrees and with humidity between 47% and 79% (6). Obviously, once the virus has spread, it tends to expand globally, that's why, at present, the involvement of the infection is almost global.

Humidity, temperature and atmospheric pressure are factors on which the dew point depends. The dew point indicates the temperature at which the relative humidity of the air reaches 100% saturation. Relative humidity represents the percentage of water vapor in the air at a given temperature, that changes when the air temperature changes. A higher dew point indicates a higher moisture content for the air. The air is saturated when the dew point corresponds to the air temperature. The dew point never exceeds the air temperature. The lower the dew point, the less water, in the form of vapor, can be contained in the air and *vice versa*. When temperatures drop, relative humidity increases. High relative humidity of the air occurs when the air temperature approaches the dew point value.

Sterling in the 1985 described the optimal relative humidity range, in which most viruses survive. In particular, the influenza virus survives better in aerosols of low relative humidity (<50%). Some viruses prefer a high relative humidity, whereas some other viruses a low relative humidity, so there is an average humidity range between 50% and 70% in which the viral population is minimal (7). The above would lead us to think, optimistically, that the transmission of the infection will decrease with the increase in temperatures and humidity typical of the late spring and summer in temperate climate countries.

However, a crucial point is the progressive changing of the climate in countries with a temperate climate. This climatic transformation depending on the Earth's spin axis drift, determines strong thermal excursions which destabilize the climate and could favor the survival of the virus.

Recently, NASA scientists have defined three main processes responsible for the Earth's spin axis drift, among which the melting of the global cryosphere, in particular Greenland, over the course of the 20th century is one of the most important (8). The melting of the glaciers could be the consequence of climate changes.

According to the Intergovernmental Panel on Climate Change (IPCC), the United Nations climate committee, anthropogenic warming has led to major climate changes in climate zones. In particular, it led to an increase in dry climates and a decrease in polar climates. Furthermore, continuous warming should lead to new warm climates in tropical regions and move climatic zones to medium-high latitudes and to regions with higher altitudes. Climate change has always influenced the Earth, but until a few centuries ago they were slow, related to known natural phenomena such as the oscillations of the Earth's axis and volcanic phenomena. However, these changes have been much more rapid in recent years. According to the report of the IPCC on Earth-climate interactions, the influence of man on climate change is undoubted and current climate change is such as to compromise the adaptability of the living being (9).

Furthermore, researchers reported that the alarm is particularly serious for Italy, which is heating up faster

than the global average of the planet. In particular, the warming of global Italy would seem to be one and a half times that of the Earth's average and twice that of the entire globe (10). The storms and floods that have occurred in Italy in recent years are clear evidence of these climate changes. In addition, not by chance in Italy SARS-COV-2 has spread particularly in the North of the country where the cities with the highest industrial pollution are concentrated (Bergamo, Milano, Brescia).

Consequently, an immediate intervention on environmental policy through massive environmental operations against pollution and the promotion of alternative energies are mandatory also to prevent pandemics such as that of COVID-19.

Certainly, it is not possible, in the short term, to change the global climate, but since environmental conditions can vary substantially between indoor and outdoor (4, 5), it is absolutely possible to change the indoor climate by changing the humidity and temperature, especially in crowded places, workplaces, schools, hospitals especially intensive care units but also at home. This type of strategy could also benefit in the short term, above all in terms of preventing viral infections and viral spread.

Unfortunately, while outdoor relative humidity is higher in winter, indoor relative humidity is much drier due to heating. Hence, exposure to cold outside air and indoor dry air increases, again, the transmission of the virus. On the contrary, incidence of viral acute respiratory diseases seems to be lower among occupants of building with low humidity levels. Indeed, previous studies demonstrated significant reduction in respiratory infections among occupants of humidified buildings (7).

In healthy people and in physiological conditions, inhaled air reaches approximately 31–34 °C and 90–100% relative humidity by the time it leaves the nasopharynx. In the respiratory airways, cold air dries airflows through the respiratory tract and inhibits mucociliary clearance. In addition, at low humidity the respiratory droplets evaporate, decrease their size and increase the ability to travel further. These phenomena increase again the possibility of viral transmission (7).

According to Sterling, the potential cause of the reduction in respiratory infections is probably due to an

increase in the settling rate of aerosols at high humidity (7). So, a therapeutic strategy against Covid-19 could be creating in the upper and lower respiratory tract conditions of humidity that are unfavorable to the virus. So, from a clinical point of view, humidification of the lower respiratory tract becomes particularly useful in patients hospitalized for pneumonia, especially when oxygen therapy is needed (11).

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