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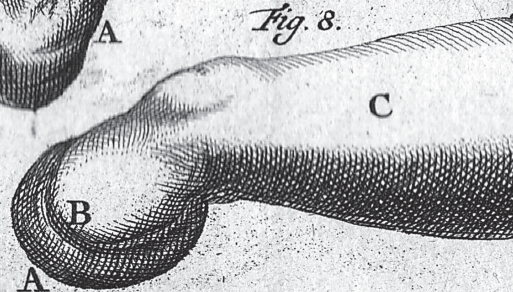
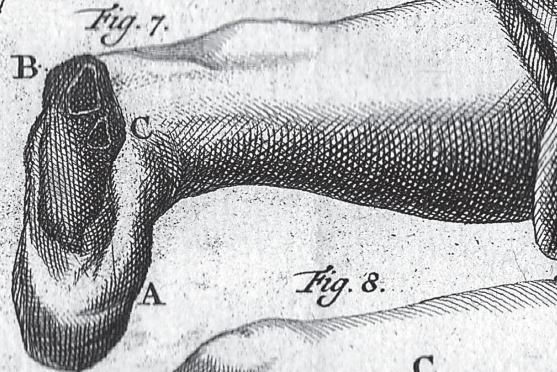
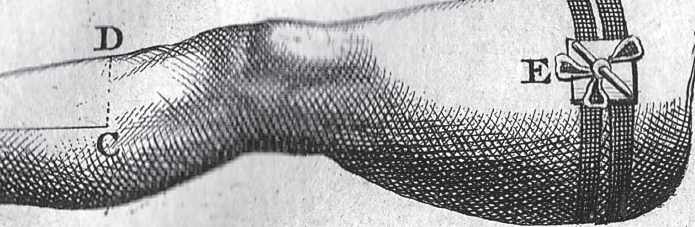
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# Hypersensitivity Pneumonitis High-resolution Computed Tomography Findings, and Their Correlation with the Etiology and the Disease Duration

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**Key words:** Hypersensitivity pneumonitis – Computed tomography – Etiology

**Abstract:** Hypersensitivity pneumonitis (HP) is an immune-mediated diffuse parenchymal lung disease induced by inhaled antigens. High-resolution computed tomography (HRCT) is widely used in the diagnosis and follow-up of patients and determining the progression and prognosis of the disease. In this retrospective study, 45 consecutive patients with the final diagnosis of HP, seen at a large tertiary care center during a period of 4 years, were included and their HRCT findings were evaluated. The most common HRCT findings were ground glass opacity and reticulation. Some HRCT patterns were detected more severely in bird fanciers in comparison with other etiologies. There is no “gold standard” for the diagnosis of HP. HRCT findings play an important role in hypersensitivity pneumonitis diagnosis and CT scan also help to define the severity of hypersensitivity pneumonitis injuries. In our study, reticulation and ground glass opacity were the most common findings in HRCT of patients with HP. We also find that patients with avian contacts had a significantly higher rate of fibrosis.

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## Introduction

Hypersensitivity pneumonitis (HP), also known as extrinsic allergic alveolitis, is an immune-mediated diffuse parenchymal lung disease, induced by repeated inhalation of an antigen in a sensitized host (Hanak et al., 2007; Mooney et al., 2013). The number of known antigens is extensive and increasing and those causing farmer's lung and pigeon breeder's/birds fancier's diseases are the most common (Hanak et al., 2007; Rosenman, 2009; Franks and Galvin, 2010). HP is clinically classified as acute, subacute and chronic. The most common forms of the disease are acute and subacute with only a low percentage progress to chronic forms (Walsh et al., 2012). Patients with chronic HP have radiologic and histopathologic evidence of fibrosis (Cormier et al., 2000). In all forms of HP, there is no "gold standard" test or approach. One widely used criteria include: 1) symptoms compatible with HP, 2) evidence of an appropriate antigen exposure by either history or an antibody testing result, 3) presence of episodic respiratory symptoms that correlates with recurrent antigen exposure, 4) imaging findings compatible with HP, 5) a lymphocytosis on BAL, and 6) histopathologic features compatible with HP (Lacasse et al., 2012; Mason et al., 2016; Pereira et al., 2016; Vasakova et al., 2017; Dias et al., 2018). The radiographic chest X-ray has always been important in diagnosis, however, the recently developed high resolution computed tomography (HRCT) has become a highly informative diagnostic parameter, which is more sensitive and the normal HRCT is a strong evidence against the diagnosis of active HP (Cormier et al., 2000; Franks and Galvin, 2010). HRCT is widely used in the follow-up of patients, determining the treatment responses and prognosis of the disease (Walsh et al., 2012; Dias et al., 2018). The outcome of HP is variable and depends on clinical and radiological presentation (Vasakova et al., 2017). It seems that HRCT findings are sufficient for the diagnosis of HP in the setting of antigen exposure and exclusion of other differential diagnoses. The characteristic HRCT features of HP are ground glass opacities, centrilobular nodules, and mosaic attenuation. Other findings include consolidation, reticulation, fibrosis, emphysema, and cysts. These findings have been described mostly in middle and upper lung zones and HP classically spares the pulmonary bases. Ground glass opacities represent active inflammation or fibrosis and are predominant in acute and subacute forms. Centrilobular nodules are mostly detected in subacute form. However, they can also be associated with fibrosis in chronic forms which mainly have ground glass opacity. Mosaic attenuation is described in chronic HP and is more apparent in expiratory scans. Reticulation is the most frequent finding in chronic HP. Consolidation is not the main abnormality in HP HRCT and may represent areas of organizing pneumonia or superimposed infections or acute exacerbation (Adler et al., 1992; Cormier et al., 2000; Silva et al., 2007; Rosenman, 2009; Franks and Galvin, 2010; Akira and Suganuma, 2014; Lynch, 2016; Pereira et al., 2016; Vasakova et al., 2017; Dias et al., 2018).

## Material and Methods

### *Study population*

In this retrospective study, 45 consecutive patients with the final diagnosis of HP seen at our tertiary lung center, were included. HP diagnosis was based on clinical history including chronic respiratory symptoms and history of antigen exposure, abnormal pulmonary function tests, and radiologic findings and compatible HRCT reviewed by thoracic radiologists, exclusion of other mimic diseases, bronchoalveolar lavage (BAL), and lung biopsy. Data from all patients were collected by questionnaires and physician review. If there were any further question or unclear history, patients were contacted again. All the patients signed the written consent forms. Patients' demographics, occupation, history of tobacco, antigen exposure, lab tests, pulmonary function tests, and HRCT findings reviewed by thoracic radiologists were recorded. Antigens exposure determined by clinician were classified to plants antigens, birds related antigens, pets, chemical materials or unknown origin.

### *Radiographic assessment*

HRCT was performed using 16-slice multi-detector CT scanner (SOMATOM Definition Flash, Seimens Healthcare, Forchheim, Germany). All patients were examined with the supine position in a suspended deep inspiration from lung apices to lung bases with standard acquisition 200 mA, 120 kVp, 1.25 mm collimation and 10 mm intervals. No intravenous contrast was used in any of the included HRCT studies. Ground glass opacity was defined as increased lung opacity without obscuring pulmonary architecture or pulmonary vessels (Austin et al., 1996). Centrilobular nodules were defined as small round opacities measuring less than 7 mm in the center of pulmonary secondary lobules (Austin et al., 1996; Cormier et al., 2000). Mosaic attenuation was defined as a patchwork of areas with different attenuation (Figure 1). Consolidation was defined as an area of increased parenchymal opacity obscuring vessels and airway margins (Austin et al., 1996). Reticulation was considered as linear opacities forming a mesh appearance (Austin et al., 1996). Emphysema and cysts were defined as areas of low attenuation without and with thin wall respectively (Austin et al., 1996; Cormier et al., 2000). Honeycombing was defined as a subpleural cluster of well-defined cystic airspaces of different sizes (Figure 2) (Austin et al., 1996). Traction bronchiectasis was also defined as irregular bronchial dilation around or within abnormal parenchyma (Figure 3) (Austin et al., 1996; Cormier et al., 2000; Tateishi et al., 2011). Fibrosis was defined as the presence of honeycombing, linear irregular opacities, or traction bronchiectasis (Hanak et al., 2008). The presence of HRCT findings was determined by two thoracic radiologists separately and the distribution was also specified in lung zones.

HRCT images were reviewed by two thoracic radiologists and the results were achieved by consensus.

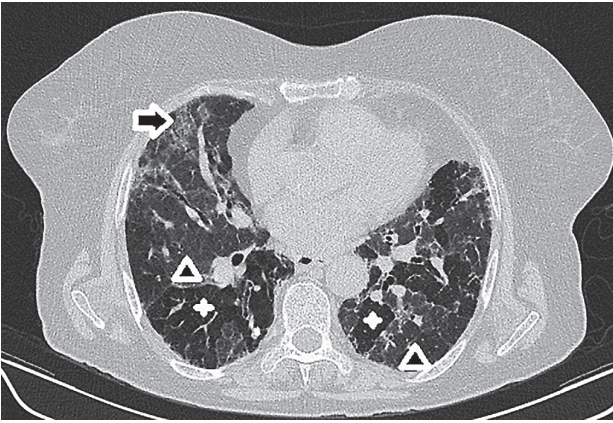


Figure 1 – Hypersensitivity pneumonitis. Axial high-resolution computed tomography demonstrates mosaic attenuation, “head cheese sign”. Areas with normal parenchyma (arrowhead), areas of air trapping (four-point star), and ground glass opacity (arrow).

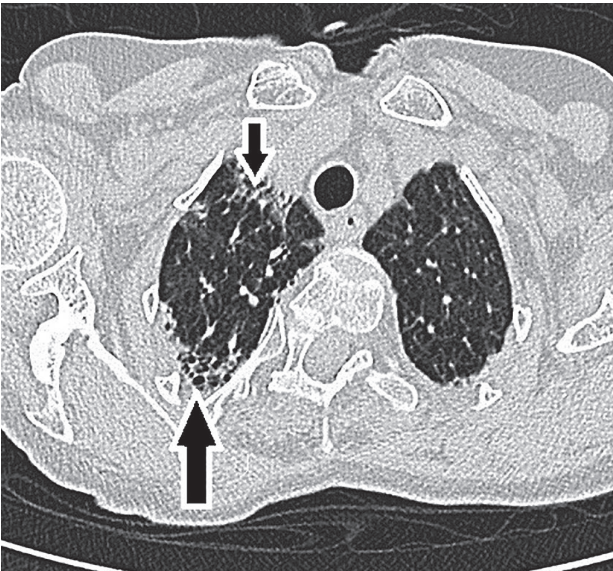


Figure 2 – Hypersensitivity pneumonitis. Axial high-resolution computed tomography demonstrates honeycombing.

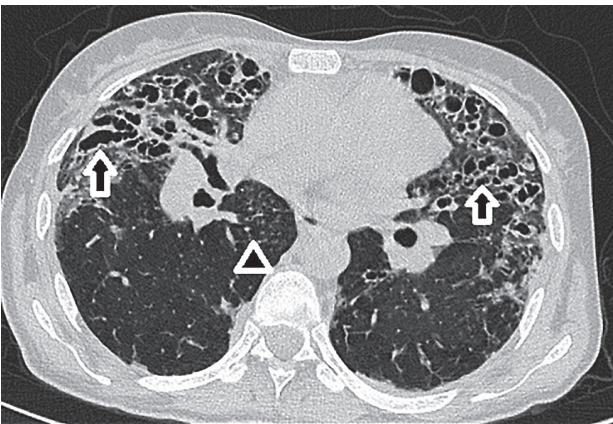


Figure 3 – Hypersensitivity pneumonitis. Axial high-resolution computed tomography demonstrates traction bronchiectasis (arrow), centrilobular nodules (arrowhead).



*Statistical analysis*

Analyses were performed with Statistical Package for Social Sciences (SPSS) software version 19. Categorical variables were described by percentages and were compared by Chi-square or Fisher's exact tests as appropriate. Continuous variables were described by means and standard deviation and were evaluated by *t*-test. A *p*-value < 0.05 was considered as statistically significant. Lungs were considered as six zones (upper, middle and lower zones on each side).

**Results**

In this study, 45 patients (23 males, 22 females) with hypersensitivity pneumonitis diagnosis were enrolled. The average age of patients was  $53.24 \pm 18.69$  (range 20–86) years old. The mean time from onset of clinical symptoms was  $7.47 \pm 6.18$  (range 1–28) years. The prevalence of HRCT findings was 93.3% for reticulation and ground glass opacity, 75.6% for fibrosis, and 71.1% for air trapping and traction bronchiectasis as classified in Table 1. The predominant findings on HRCT were reticulation and ground glass opacity. Reticulation was mostly diffuse in lungs in 76.1%. Reticulation was diffuse bilateral in 50% of patients, in 6.2% of patients ground glass opacity was bilateral in lower lobes and in 6.2% was bilateral in the upper lobes, the other patients did not have specific distribution. There was no significant difference in HRCT features between males and females. In comparison between patients less than 50 years of age and patients with older age than 50 years old, there were significant differences in air trapping and traction bronchiectasis. Air trapping was more common in patients older age than 50 years

**Table 1 – High-resolution computed tomography findings prevalence in 45 patients with hypersensitivity pneumonitis**

HRCT feature	Cases	Prevalence (%)
Reticulation	42	93.3
Ground glass opacity	42	93.3
Fibrosis	34	75.6
Air trapping	32	71.1
Traction bronchiectasis	32	71.1
Ground glass nodules	27	60.0
Solid nodules	6	13.3
Consolidation	28	62.2
Emphysema/cysts	17	37.8
Honeycombing	16	35.6
Mosaic attenuation	26	57.8
Costophrenic angle sparing	22	48.9
Pleural reaction	6	13.3
Lymphadenopathy	20	44.4

HRCT – high-resolution computed tomography

**Table 2 – Etiologic various exposure in 45 patients with hypersensitivity pneumonitis**

<b>Etiology</b>	<b>Cases</b>	<b>Prevalence (%)</b>
Unknown	13	28.9
Molded and agriculture-related antigen	11	24.4
Birds	7	15.6
Other pets	2	4.4
Various chemicals	12	26.7

old (95.7% vs. 45.5%,  $p$ -value < 0.001). Traction bronchiectasis was also more common in patients older age than 50 years old (91.3% vs. 50%,  $p$ -value = 0.002). Of these 45 patients, there was no known etiology in 28.9%, and so on 26.7% had contacts with agriculture-related antigens, 15.6% had contacts with birds, 4.4% were exposed to the other pets' antigens, and 28.9% were exposed to different types of chemicals (Table 2). In our study, patients with avian contacts had a significantly higher rate of fibrosis in comparison with other etiologies (81.6% vs. 42.9%,  $p$ -value = 0.028). There was significant higher rate of traction bronchiectasis and honeycombing in patients with avian contacts compared to other etiologies (78.9% vs. 28.6%,  $p$ -value = 0.015 and 42.1% vs. 0%,  $p$ -value = 0.032, respectively). There were no significant differences in HRCT patterns of other etiologies. In comparison of patients with disease duration more than 5 years and patients with duration less than 5 years, there was significant higher rate of air trapping and emphysema/cysts in patients with disease duration more than 5 years (85.7% vs. 58.3%,  $p$ -value = 0.043, and 52.4% vs. 25%,  $p$ -value = 0.05). Fibrosis had a significantly higher prevalence in patients with disease duration more than 5 years (95.2% vs. 58.3%,  $p$ -value = 0.004). There was a higher rate of traction bronchiectasis in patients with disease duration more than 5 years compared to patients with shorter duration of disease.

## Discussion

In this study, we describe HRCT findings in 45 patients with a final diagnosis of HP. Dias et al. (2018) reviewed the essential features of CT in hypersensitivity pneumonitis and discussed HP as a complex disease with reticular opacities, traction bronchiectasis, and architectural distortion as main CT findings. They also mentioned that in advanced stages, HP may resemble nonspecific interstitial pneumonia (NSIP) or usual interstitial pneumonia (UIP) but evidence of small airways involvement such as centrilobular ground-glass opacities, peribronchovascular involvement, upper lobe predominance and mosaic pattern can help the diagnosis (Dias et al., 2018). In our study, the predominant findings on HRCT were reticulation and ground glass opacity and our study confirmed the previous studies that ground glass opacity and findings of fibrosis, namely

reticulation, architectural distortion, and traction bronchiectasis with or without honeycomb change are most common (Cormier et al., 2000; Tateishi et al., 2011; Walsh et al., 2012; Vasakova et al., 2017). Vasakova et al. (2017) have described emphysema in patients with HP. Cormier et al. also confirm previous reports that emphysema is a long-term outcome in patients with farmer's disease (Erkinjuntti-Pekkanen et al., 1998; Cormier et al., 2000). In our study, patients with disease duration more than 5 years had a higher rate of emphysema and air trapping. Air trapping was more common in patients with older age than 50 years old.

Walsh et al. (2012) implied that HRCT is increasingly used to assess chronic hypersensitivity pneumonitis. They also suggested that HRCT patterns are superior to pulmonary function tests for mortality prediction and extensive traction bronchiectasis strongly predicts poor survival in chronic hypersensitivity pneumonitis (Walsh et al., 2012). Some other studies also mentioned that fibrosis extent could be a high confidential prognostic factor (Hanak et al., 2008; Mooney et al., 2013; Pereira et al., 2016). In our study, fibrosis had a significantly higher prevalence in patients with disease duration more than 5 years and there was a higher rate of traction bronchiectasis in patients with disease duration more than 5 years compared to patients with shorter duration of disease.

Hanak et al. (2007) notified that avian antigens were the most common etiology of HP (34%) in their study and in 25% of the patients inciting antigen could not be identified. Inciting antigens are different in various geographic regions depending on climate, culture, socioeconomic status, and occupational factors. In England, the most common etiology of HP has been reported avian antigens, versus molds in Japan (Hendrick et al., 1978; Bourke et al., 2001; Hanak et al., 2007). In our study, the most common antigens causing HP were molded and agriculture-related antigens (24.4%), birds' antigens were responsible in 15.6% of patients, and 28.9% had unknown etiology.

In our study, patients with avian contacts had a significantly higher rate of fibrosis in comparison with other etiologies. There was a significantly higher rate of traction bronchiectasis and honeycombing in patients with avian contacts compared to other etiologies. There were no significant differences in HRCT patterns of other etiologies. This may suggest that HP with avian contacts may have more severe HRCT patterns and subsequently poorer prognosis which needs further evaluation and studies to be proved.

## Conclusion

HRCT findings are an important part of hypersensitivity pneumonitis diagnosis. CT can also help to define the severity of hypersensitivity pneumonitis injuries. In this study, we found that reticulation and ground glass opacity were the most common findings in HRCT of patients with HP. It was also noted that air trapping, emphysema, and fibrosis had a higher rate in patients with disease duration more than 5 years. Patients with avian contacts had a significantly higher rate of fibrosis in



comparison with other etiologies but there were no significant differences in HRCT patterns of other etiologies.

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# Endovascular Treatment of a Life-threatening Blunt Thoracic Aortic Injury in Polytraumatized Patients – A Single Center Experience

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**Key words:** Endovascular treatment – Blunt thoracic aortic injury – Polytrauma

**Abstract:** A retrospective analysis of our group of patients, efficacy, safety and the results of endovascular treatment of descending thoracic aorta by using stentgraft implantation in polytraumatized patients. In the period between 6/2006 and 2/2020, in the processing of data, we analysed retrospectively patients with polytrauma diagnosed with thoracic aortic rupture or transection (TAT) and treated with multiple injuries. Clinical characteristics, complications, pathological features, and hospital follow-up data were retrieved from our group. In our group of 28 polytraumatized patients referred to our Trauma Centre with current TAT, all 28 patients with such a thoracic aortic injury were treated by using thoracic stentgraft implantation. In our group of patients, the average Injury Severity Score (ISS) was 22 for women (min 19, max 27) and 26 for men (min 17, max 41), respectively. We reached 100% technical implantation success rate with our patients. In our group, we had 30-day mortality of 10.7% (3 patients) and the in-hospital mortality was 17.8% (5 patients). Surviving patients had calculated ISS score of 25 (min 17, max 41); dead patients had an ISS score of 28 (min 19, max 34) –  $p \leq 0.05$ . Endovascular treatment of TAT, as a minimally invasive and effective procedure with rapid bleeding control, may increase survival chances for severely compromised polytraumatized patients in the context of multiple-organ damage and the need for a major cardio-vascular surgery.

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## Introduction

The term polytrauma is defined in literature in different ways and according to different aspects. Polytrauma typically refers to severely injured patients with two or more significant traumatic injuries (Border et al., 1975). Various scoring systems are used worldwide for the clear objectification of injury severity, most commonly the Injury Severity Score (ISS) and the Abbreviated Injury Scale (AIS). An Injury Severity Score (ISS)  $\geq 16$  and an Abbreviated Injury Scale (AIS)  $\geq 3$  in at least two different body regions are in general the globally accepted values for the definition of “polytrauma” (Boyd et al., 1987; Butcher et al., 2014). The values are based on a description for the prediction of mortality of injured patients above 10% (Boyd et al., 1987). Polytrauma is the most common cause of death of patients younger than 40. The incidence of deaths associated with traumas in developed countries is 60–80/100,000 inhabitants. The main cause is traffic accidents. The most common cause of death in traffic accidents is brain injury and another fatal cause of death is a deceleration blunt aortic injury. Thoracic aortic rupture or transection (TAT) carries an overall mortality of 90% (Parmley et al., 1958). Approximately 15% of traffic accidents are associated with a thoracic aortic injury (Smith and Chang, 1986) and the injury ranks second as a cause of death in fatal traffic accidents. 85% of patients with a thoracic aortic injury die before being transported to the hospital and 15% have variable survival rates, of which about 2% of patients suffer from a chronic pseudoaneurysm (Smith and Chang, 1986; Reardon et al., 1997). More theories

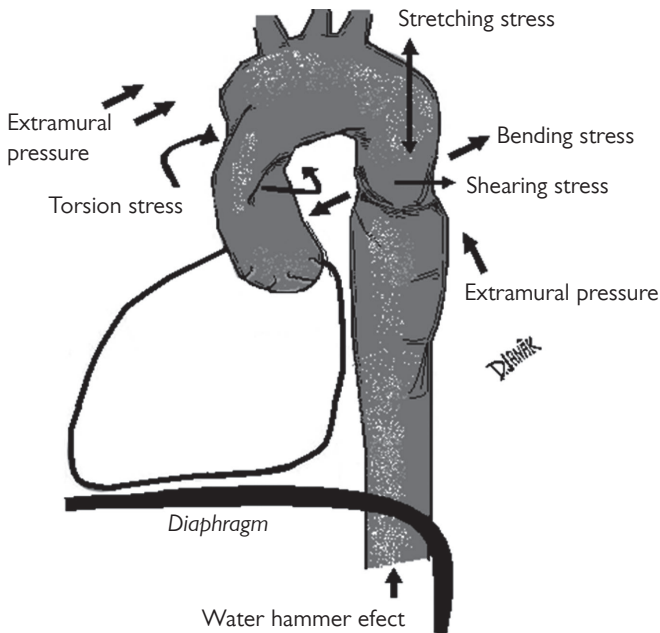


Figure 1 – Mechanism of TAT (thoracic aortic rupture or transection).

propose that TAT results from a combination of mechanisms including shear, torsion, bending and stretching stress (Figure 1). The compression force on the sternum and abdomen cause the mediastinum to displace upwards, often termed a “shovelling effect”. As the chest is compressed, the heart is squeezed between the sternum and the spine forcing blood from the heart into the aorta and creating a dramatic rise in the blood pressure of the aorta and pulmonary trunk. In addition, it is anticipated that at the level where the aorta passes through the diaphragm, it can kink and occlude the blood flow through the aorta and contribute to the rise in aortic blood pressure. This sudden occlusion of the blood flow can lead to high-pressure waves in the aorta due to a “water-hammer” effect. The “shovelling effect” displaces the heart and aortic arch upwards, while the descending aorta, which is tethered to the spine via the lungs remains fixed (Richens et al., 2002).

## Methods

In the period between 6/2006 and 2/2020, in the processing of data, we analysed retrospectively patients with polytrauma diagnosed with thoracic aortic rupture or transection (TAT) and treated with multiple injuries. These polytraumatized patients were transported to our Trauma Centre, which, together with two other trauma centres, provides the catchment area of the whole Prague and its vicinity comprising 2600,000 inhabitants. During this period, we dealt with 28 polytraumatized patients diagnosed with TAT who were referred to us primarily or who were referred from lower trauma centres. All patients were admitted through the emergency department; diagnosis and life-saving procedures (life-threatening to small lesions) were performed in cooperation with traumatologists and general surgeons, when using Advanced Trauma Life Support (ATLS) protocol, and all patients were treated with a full-body CT (computed tomography) scan with contrast media based on a trauma protocol. A CTA (computed tomography angiography) examination in these patients confirmed multiple injuries and TAT, and the simultaneous computer reconstruction of images helped to plan endovascular and surgical procedures, if any. All 28 patients in our group were treated by using thoracic endovascular stentgraft implantation. The procedures were performed by interventional radiologists in the radiological room with cardiovascular surgeons and anesthesiologists. In our Trauma Centre, the team of vascular surgeons and interventional radiologists has a 24-hour duty. Stentgrafting was performed through the femoral arteries. The device was inserted by either a puncture or surgical technique (we used during surgical technic low dose mini-heparinization). The procedure was conducted under DSA (digital subtraction angiography) control and the stentgrafts had 10% oversizing. The implantation was performed without the administration of heparin and with antibiotic prophylaxis. In our group of patients there was no anatomical contraindication from the point of view of insufficient proximal landing zone for stentgraft implantation. A follow-up CTA examination of the thoracic aorta is conducted immediately after thoracic aortic stentgraft implantation

during hospitalization, then after 1 year and after 2–3 years. In addition to CTA examinations, we also perform contrast-enhanced ultrasound (CEUS) examinations 3 months after stentgraft implantation. In our group of patients with implanted stentgraft who were treated in a broad time period (between 6/2006 and 2/2020) we have patients with a wide variety of examinations, with 1-year, 2-year, 3-year intervals but also patients with more than 10-year intervals after implantation; the longest period is 13 years. Some patients are foreign nationals and are no longer kept in our records. All our patients registered during long-term follow-up examinations did not show any complication due to the implanted thoracic stentgraft. As of January 2020, we do not have any living patients with the evidence of stentgraft migration, endoleak or significant aortic dilatation in the thoracic region of the aorta (one patient with endoleak Ia after re-intervention, recently without endoleak).

**Table 1 – Basic patient information, mechanism of injury and type of injury**

All patients	n=28
Stentgrafts	29
Sex – female	8 (28.5%)
Sex – male	20 (71.5%)
Sex – female – age	50.8 years (min 25, max 84)
Sex – male – age	32.8 years (min 19, max 73)
Injury Severity Score (ISS) – female	22 (min 19, max 27)
Injury Severity Score (ISS) – male	26 (min 17, max 41)
Mechanism of injury	numbers of patients (%)
Car accident	14 (50.0%)
Motorcycle accident	3 (10.7%)
Pedestrian	1 (3.5%)
Ski accident	1 (3.5%)
Train accident	1 (3.5%)
Fall from 5 m	1 (3.5%)
Fall from 10 m	6 (21.4%)
Fall from 20 m	1 (3.5%)
Associated injuries	numbers of patients (%)
Head	12 (42.8%)
Neck	3 (10.7%)
Chest	18 (62.4%)
Abdominal	8 (28.5%)
Retroperitoneal space	4 (14.2%)
Abdominal + retroperitoneal space	2 (7.1%)
Pelvis	10 (35.7%)
Limbs	18 (62.4%)
Pneumothorax	10 (35.7%)
Cervical spine fractures	3 (10.7%)
Thoracic spine fractures	3 (10.7%)
Lumbal spine fractures	6 (21.4%)



## Result

Of the 28 patients (71.5% males with mean age 32.8 years [min 19, max 73 years] and 28.5% women with mean age 50.8 years [min 25, max 84 years]), the cause of TAT polytrauma in 14 patients was due to a traffic accident (vehicle occupants), in 3 patients it was due to a motorcycle accident, 1 – pedestrian, 1 – ski accident, 1 – fall from the height of 5 m, 6 – fall from the height of 10 m, and 1 – fall from the height of 20 m (Table 1). In our group of patients, the average ISS score was 22 for women (min 19, max 27) and 26 for men (min 17, max 41), respectively. In our group of 28 polytraumatized patients referred to our Trauma Centre with current TAT (Figure 2), all 28 patients with such a thoracic aortic injury were treated

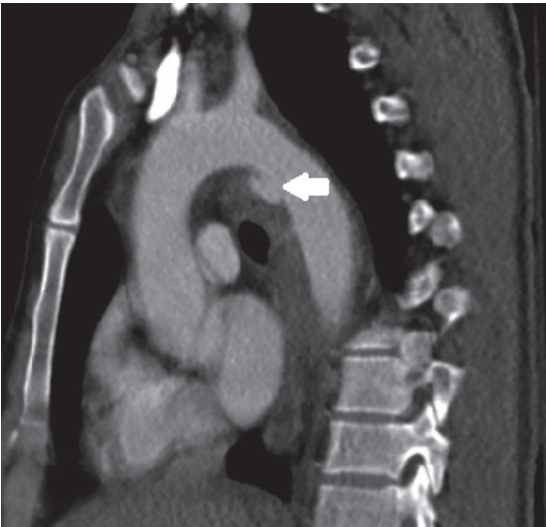


Figure 2 – Thoracic aortic rupture or transection (arrow) after car accident.



Figure 3 – TAT (thoracic aortic rupture or transection) after implantation of thoracic stentgraft.

by using thoracic stentgraft implantation (Figure 3). In 2 patients, simultaneous surgical reconstruction of the carotid bed and the left arteria subclavia was necessary (a hybrid procedure: stentgraft + aortal debranching). This approach is especially necessary for complex arch and descendent aortic lesions. In this case, the cooperation of cardio surgeons and radiologists is necessary. The time period from the first contact of the patient in our Trauma Centre to the initiation of the thoracic stentgraft implantation was 126 min on average (in 2006, the average

**Table 2 – Stentgrafts and procedures details and complications**

<b>Stentgrafts size</b>	<b>Numbers</b>
22×100 mm	1
20×105 mm	2
22×105 mm	2
24×105 mm	4
24×115 mm	1
24×150 mm	1
26×105 mm	6
26×134 mm	2
28×140 mm	1
28×155 mm	2
32×140 mm	1
32×155 mm	2
32×160 mm	1
34×77 mm	1
34×161 mm	2
Femoral approach	28 (100%)
Percutaneous access	22 (78.5%)
Surgical access	6 (21.4%)
Left subclavian artery (LSA) coverage	10 (35%)
Revascularization of LSA	2 (7.1%)
Hybrid procedure (stentgraft + surgery)	2 (7.1%)
Emergency department (first contact) to implantation (min)	126 min (min 40, max 360)
<b>Complications</b>	<b>Numbers (%)</b>
Stentgrafts failure	1 (3.5%)
Endoleak	1 (3.5%)
Pseudoaneurysm following a. puncture	1 (3.5%)
Femoral wound complications	3 (10.7%)
Postimplantation mortality (<30 days)	3 (10.7%)
Postimplantation mortality (in hospital)	5 (17.8%)
<b>Cause of death</b>	<b>Numbers (%)</b>
MODS	3 (10.7%)
Brain injury	1 (3.5%)
Stentgrafts failure/bleeding/surgery	1 (3.5%)

MODS – multiple organ dysfunction syndrome

time was 180 minutes and in 2019 75 minutes). During this time period, further life-saving procedures for concomitant life-threatening injuries and simultaneous post-examination and planning procedures for thoracic stentgraft implantation were carried out. No patients died before stentgraft implantation. The implantation was 100% from femoral artery (78.6% percutaneous vs. 21.4% surgical techniques). We used COOK-Zenith stentgrafts for patients (Table 2). We reached 100% technical implantation success rate with our patients. In one case, the stentgraft seal failed early with subsequent bleeding and the need for surgical replacement of the thoracic aorta (subsequently with early death of the patient). In one case (patient with very anatomically unfavourable finding), post-implantation Ia endoleak occurred with the necessity of reintervention by secondary stentgraft implantation. In one case we encountered femoral artery pseudoaneurysm after a percutaneous technique and in three cases we had an early complication in the groin area after the implantation surgery. In our group, we had 30-day mortality of 10.7% (3 patients) and the in-hospital mortality was 17.8% (5 patients). In 3 patients, the cause of death was multiple organ dysfunction syndrome (MODS), in 1 patient it was a brain injury and in 1 patient it was a haemorrhagic shock after a direct stentgraft failure followed by thoracic aortic rupture followed with subsequent aortic surgical replacement and organ failure (Table 2). Surviving patients had calculated ISS score of 25 (min 17, max 41); dead patients had an ISS score of 28 (min 19, max 34) –  $p \leq 0.05$ . The value of mortality is given by the severity of concomitant fatal injuries.

## Discussion

Polytraumatized patients are a very specific group of patients with a high mortality. The main cause of death in these patients in developed countries is road accidents. TAT is a significant negative factor affecting the patient condition. Around 80–90% of patients with TAT die at the scene of the accident and of those who do reach a hospital, 15–20% of patients survive (Dosios et al., 2000). The first surgical treatment for blunt traumatic rupture of the thoracic aorta was described by Passaro and Pace in 1959. Nowadays, thoracic stentgrafts (TEVAR) are used for clear diagnoses such as thoracic aneurysm and penetrating aortic ulcer. However, endovascular treatment is becoming an emergent option for highly compromised TAT patients with increasing numbers of polytraumatized patients. At present, some experts ask whether the surgical treatment of TAT is still the “gold standard” but based on available studies (Riesenman et al., 2007; Buz et al., 2008; Chung et al., 2008) and meta-analyses (Takagi et al., 2008; Tang et al., 2008; Xenos et al., 2009) predicting a lower mortality and shorter procedure time, the solution shifts significantly towards a primary endovascular TAT treatment. An analysis of the studies is difficult and limited due to a small number of patients with different characteristics and, for example, the time prior to the commencement of procedures. The evaluated meta-analyses have drawn conclusions in favour of endovascular treatment of TAT. However, it should be noted that meta-analyses

evaluating unilaterally interpreted studies with a small number of different patient characteristics may lead to biased results compared to the actual condition. The basis for patient survival is early diagnosis, prompt pharmacological therapy (a pressure control) and urgent handling of findings. The treatment of this type of trauma is not routine in most trauma centres. Typically, approximately 3–4 cases per year are reported per trauma centre (Arthurs et al., 2009). Centres with experience in thoracic surgery, cardiac surgery and anesthesiologists are required to responsibly address polytraumatized patients with blunt aortic injuries. Experienced interventional radiologists are required in case of stentgraft implantation for TAT. Rapid and minimally invasive endovascular treatment of the aortic lesion improves the outcome of aortic injuries. Endovascular repair of traumatic aortic rupture minimizes hemodynamic instability and prevents the exacerbation of bleeding from the injured area. In young polytraumatized patients, a sharper angle and cross-section of the aortic arch than in older patients and possibly more difficult adherence of the stentgraft, should be expected. Older generations of the stentgrafts were sized for degenerative aortic diseases. These stentgrafts were more rigid and designed for larger aortic diameters. There was a threat of great instability, stentgraft migration and possible pseudocoarctation of the aorta (Azizzadeh et al., 2014; Mouawad et al., 2020). The new generation of stentgrafts is more flexible with better adherence and smaller cross-sections for smaller types of the aorta in young patients. The short- and medium-term results of endovascular treatment of thoracic aortic aneurysms are encouraging. Concerns about the subsequent complications resulting from stentgraft implantation such as stentgraft migration and collapse are relevant. Due to the development of new generations of stentgrafts with better adherence and compliance even for narrow aortas, there is an assumption of improving results of stentgraft implantation and minor complications associated with stentgraft implantation. Polytraumatized patients are a very specific group of patients with critical injuries to the CNS (central nervous system), chest, abdomen and extremities, where a fast mini-invasive TAT treatment minimally affects the patient's hemodynamic status. Patients need not undergo thoracotomy and no extracorporeal circulation is needed. Meta-analyses have shown the incidence of paraplegia after TEVAR below 1% as opposed to 7% after surgical treatment (Xenos et al., 2008; Jonker et al., 2010). Spinal cord ischemia (SCI) had always been a grievous complication of thoracic endovascular aortic repair. It often results in paraplegia, reversible or permanent. A number of patient and procedure-related factors have been shown in previous studies to be associated with the development of SCI after TEVAR, including extensive exclusion of the aorta by endografts (more than 15 cm of thoracic aorta), perioperative hypotension (mean arterial pressure) < 70 mm Hg (Chiesa et al., 2005), previous or concomitant thoracic aortic repair and coverage of the left subclavian artery (LSA). Arm ischemia after unprotected LSA occlusion occurred in 20%. To prevent or minimize this complication, widely employed prophylactic measures include cerebrospinal fluid (CSF) drainage, arterial pressure

augmentation and LSA revascularization (Bobadilla et al., 2013). To prevent spinal cord ischemia, we used short stentgrafts (up to 15 cm in 22 patients – 78.5%), prevent LSA revascularization (2 patients – 7.1%), coverage of the left subclavian artery only in 10 patients – 35.7%, arterial pressure augmentation and corticosteroids. Young patients have a lower incidence of atherosclerosis and a lower risk of spinal cord ischemia. Rizvi et al. (2009) performed a meta-analysis on 3,365 patients from 51 observational studies on TEVAR procedure with or without left subclavian coverage. Results from 8 studies showed a non-significant increase in the risk of SCI with LSA coverage (Rizvi et al., 2009). Recently, Patterson et al. (2014) conducted a meta-analysis on 1,002 TEVAR patients from the Medtronic Thoracic Endovascular Registry (MOTHER) data derived from five clinical trials and one institutional series and reported that subclavian coverage did not increase the risk of SCI (5%) compared to those without coverage (3%) ( $p=0.16$ ). In our cohort, none of the patients with complete LSA coverage developed ischemic complaints of the left arm and no significant SCI. Planned coverage of LSA is an acceptable risk for arm ischemia and SCI in life-threatening injuries. Based on the results found in our population, we speculate that complete LSA coverage during TEVAR is relatively safe and did not lead to significant ischemic complications. However, this topic is very controversial in the available literature. The optimization of the care system with the assistance of experienced anesthesiologists, vascular surgeons and interventional radiologists improves overall care for these complicated patients. Standardized care leads to improved diagnosis and time periods leading to life-saving procedures. At present, we prefer percutaneous femoral techniques with minimal complications in our Trauma Centre. In our group of patients, we had a 100% technical success rate of implantation and the necessary coverage of subclavian artery on the left did not result in upper limb ischemia. An analysis of the mortality of polytraumatized patients with TAT based on our data and experience shows that systemic complications and complications resulting from damage to the brain, abdominal cavity and cardiorespiratory system, are the dominant cause of death after timely adequate endovascular treatment of the thoracic aorta. Our group of patients shows that the ISS score does not affect the technical success rate of thoracic stentgraft implantation. The results show that patients with a higher ISS score have higher mortality. The advantage of stentgraft implantation without the need for heparin is a lower risk of further bleeding. At the same time, in these mostly young patients, a post-implantation check-up of stentgrafts is necessary due to the possibility of further re-interventions, which are likely due to the expected length of time of artificial material in the thoracic aorta. This potential need for re-interventions after the endovascular treatment of TAT is a certain disadvantage.

## Conclusion

Endovascular treatment of TAT, as a minimally invasive and effective procedure with rapid bleeding control, may increase survival chances for severely compromised



polytraumatized patients in the context of multiple-organ damage and the need for a major cardio-vascular surgery. An important role in improving survival results for these patients has the need for patient centralization in large centres that have experience with this specific issue and a clearly established algorithm for timely and targeted care for these patients. The algorithm mainly includes a clearly defined targeted aortic examination and early transport, and thoracic stentgraft implantation to control bleeding.

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# Age Dependent Progression of Multiple Epiphyseal Dysplasia and Pseudoachondroplasia Due to Heterozygous Mutations in *COMP* Gene

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**Key words:** Short stature – Multiple epiphyseal dysplasia – Pseudoachondroplasia – *COMP*

**Abstract:** Dominantly inherited mutations in *COMP* gene encoding cartilage oligomeric matrix protein may cause two dwarfing skeletal dysplasias, milder multiple epiphyseal dysplasia (MED) and more severe pseudoachondroplasia (PSACH). We studied the phenotype and X-rays of 11 patients from 5 unrelated families with different *COMP* mutations. Whole exome and/or Sangers sequencing were used for molecular analyses. Four to ten X-ray images of hands hips, knees or spine were available for each patient for retrospective analyses. Eight patients with MED have mutation c.1220G>A and 3 children with PSACH mutations c.1359C>A, c.1336G>A, or the novel mutation c.1126G>T in *COMP*. Progressive failure in growth developed in all patients from early childhood and resulted in short stature < 3<sup>rd</sup> percentile in 7 patients and very short stature < 1<sup>st</sup> percentile in four. Most patients had joint pain since childhood, severe stiffness in shoulders and elbows but

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increased mobility in wrists. Six children had bowlegs and two had knock knees. In all patients, X-rays of hands, hips and knees showed progressive, age-dependent skeletal involvement more pronounced in the epiphyses of long rather than short tubular bones. Anterior elongation and biconvex configuration of vertebral bodies were more conspicuous for kids. Six children had correction of knees and two adults had hip replacement. Skeletal and joint impairment in patients with MED and PSACH due to *COMP* mutation start in early childhood. Although the clinical severity is mutation and age dependent, many symptoms represent a continuous phenotypic spectrum between both diseases. Most patients may benefit from orthopaedic surgeries.

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## Introduction

Cartilage oligomeric matrix protein (*COMP*) encoded by *COMP* gene and synthesized by chondrocytes, is the multifunctional extracellular matrix calcium-binding glycoprotein involved in the enhancement of chondrocyte attachment, proliferation and cartilage production, maintenance, and homeostasis (Hecht et al., 2005; Merritt et al., 2006; Briggs et al., 2014; Posey et al., 2019). Mutations in *COMP* may cause two different types of severe short-limb dwarfism with autosomal dominant inheritance pattern affecting especially the epiphyses of long bones including multiple epiphyseal dysplasia (MED/EDM1, MIM132400) and pseudoachondroplasia (PSACH, MIM177170), but an overlap between both disorders was also described as a part of a continuous phenotypic spectrum (Jackson et al., 2012; Spranger et al., 2012).

In our study we analysed the clinical course of the disease and available X-rays in 11 patients from 5 unrelated families with MED/EDM1 or PSACH due to different heterozygous mutations in *COMP* gene including c.1220G>A, c.1359C>A, c.1336G>A, and a novel mutation c.1126G>T.

## Material and Methods

The study involved 11 patients from 5 unrelated families. All of them developed progressive failure in their growth from early childhood starting between the age of 2 and 7 years. This growth failure resulted in short stature < 3<sup>rd</sup> percentile in 7 patients and short stature < 1<sup>st</sup> percentile in another four (Table 1). Most patients have stiff shoulders and elbows with severely restricted range of motion. Contrary, their wrists mobility is increased. Progressive and mostly painful gait disturbances due to legs deformities with bowlegs was present in 6 children and knock knees in two. Surgical correction of lower extremities was necessary in 6 patients during childhood and a hip replacement was required in the two oldest patients (Table 1). All patients have normal cognitive functions. Due to the lack of adequate mobility, four patients are overweight, and one is obese.

Genomic DNA from all of these patients and their parents was used for whole-exome sequencing (WES) in families A, C and E. Direct Sanger sequencing of *COMP* gene exons was performed in families B and D. Exome enrichment for WES was

**Table 1 – The main clinical symptoms in 11 patients from 5 families with skeletal dysplasias due to mutations in the COMP gene for cartilage oligomeric matrix protein**

	Family A P1–4	Family B P5–8	Family C P9	Family D P10	Family E P11
Mutation in COMP gene	c.1220G>A p.Cys407Tyr	c.1220G>A p.Cys407Tyr	c.1359C>A p.Asn453Lys	c.1336G>A p.Asp446Asn	c.1126G>T p.Asp376Tyr
Skeletal dysplasia	MED	MED	PSACH	PSACH	PSACH
Age (years)	14–47	16–48	2.5	12	13
Short stature (< 3 <sup>rd</sup> percentile)	4/4	4/4	+	+	+
Very short stature (< 1 <sup>st</sup> percentile)	1/4	1/4	–	+	+
Overweight	3/4	–	–	+	obesity
↑↑ stiff shoulders	4/4	4/4	–	+	+
↑↑ stiff elbows	4/4	4/4	–	+	+
↑ laxity in wrist	4/4	4/4	–	+	+
Bowlegs	4/4	–	–	+	+
Knock knees	–	2/4	–	+	–
Hip replacement	–	2/4	–	–	–
Knee surgery	2/4	2/4	–	+	+

MED/EDM1 – multiple epiphyseal dysplasia; PSACH – pseudoachondroplasia; overweight – BMI (kg/m<sup>2</sup>) > 90<sup>th</sup> percentiles for height; obesity – BMI (kg/m<sup>2</sup>) > 97<sup>th</sup> percentiles for height



performed on individually barcoded samples using SeqCap EZ MedExome Probes (Roche, USA) and sequenced using HiSeq 2500 (Illumina, USA). Reads were aligned to the hg19 reference genome using NovoalignCS version 3.1.11.08 (Novocraft, Malaysia) with default parameters. Sequence variants in analysed samples were identified using the GATK SAMtools package (version 3.1.10.1.8). High confidence variants list (SNP qual > 100 and Indel qual > 50) was annotated using Annovar

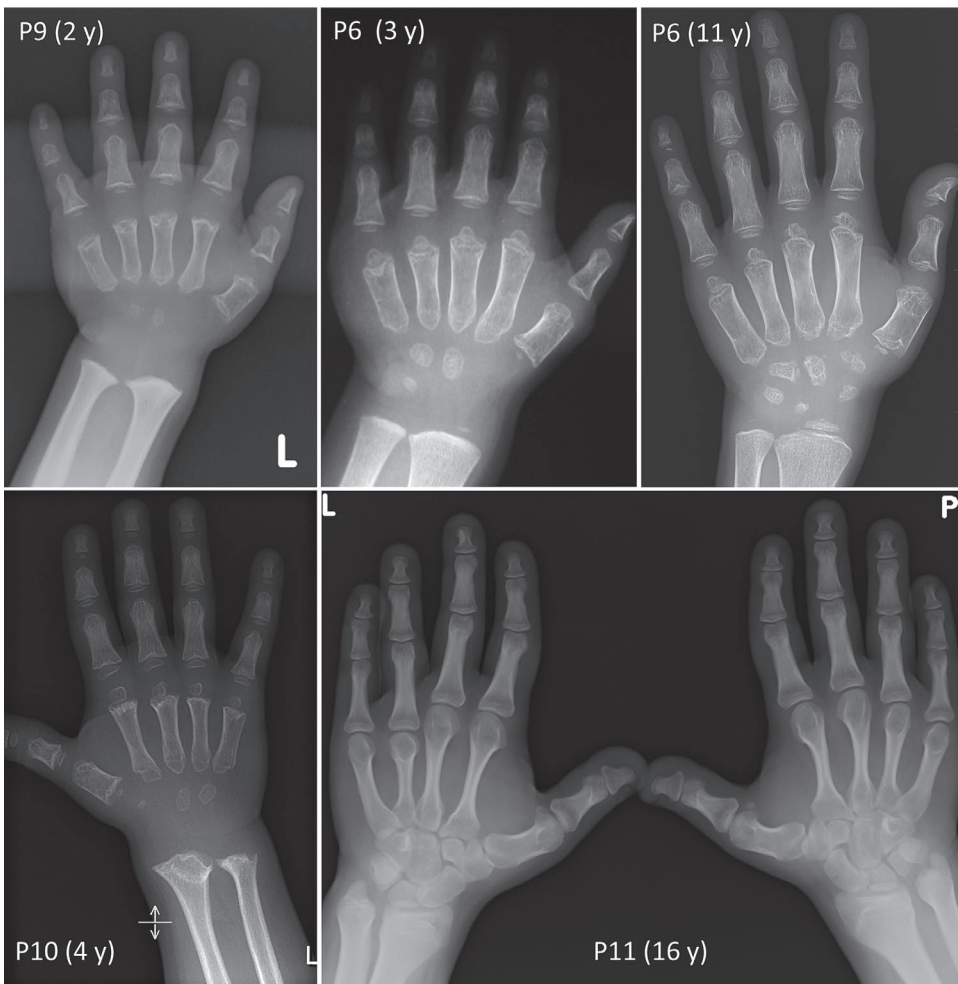


Figure 1 – X-ray survey of the hands and wrists since childhood to adulthood in patients P6, 9, 10 and 11 with multiple epiphyseal dysplasia or pseudoachondroplasia due to different mutations in *COMP* gene show deformity of the metaphyses of the shortened metacarpal bones with uneven surface. The shortening becomes more apparent after growth plate fusion. Mild shortening can be appreciated in the phalanges as well. The epiphyseal ossification centres of the metacarpal bones, distal radius and carpal bones are smaller and, in some patients, have irregular shape. The distal metaphyses of the radius and ulna have irregular contours and in adolescence show deformity that involves the radio-carpal and the distal radio-ulnar joint. The ossification is delayed.

(hg19). Due to an assumed autosomal dominant mode of inheritance, variants present in affected individuals and not present in healthy relatives were taken into consideration. Genetic background of the diagnosis of MED was reviewed and genes associated with this disorder were considered first. Presence of candidate variants was confirmed by Sanger sequencing and segregation of the variant in the whole family was performed. Four to ten X-ray images of various bones and joints from different orthopaedic departments were available for each patient.

The study was approved by the Ethics Committee of the General University Hospital in Prague and was conducted in agreement with institutional guidelines. Written informed consent for molecular analyses was obtained from all patients or their parents.

## Results

Molecular analyses in 8 patients with MED/EDM1 from families A and B revealed a dominantly inherited mutation c.1220G>A (p.Cys407Tyr) in *COMP* gene. *De novo* mutations c.1359C>A (p.Asn453Lys), c.1336G>A (p.Asp446Asn) and a new mutation c.1126G>T (p.Asp376Tyr) were found in another three patients from families C, D and E (Table 1).

In all patients, X-rays of the hands, hips and knees showed age-dependent progression of epiphyseal changes, more pronounced in the epiphyses of long than short bones. Anterior elongation and biconvex configuration of vertebral bodies

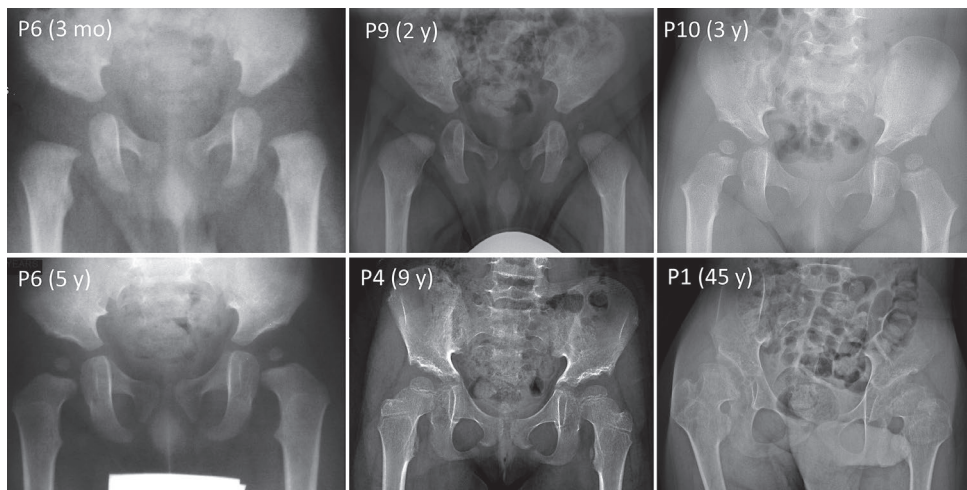


Figure 2 – X-ray survey of the hip joints since childhood to adulthood in patients P1, 4, 6, 9 and 10 with multiple epiphyseal dysplasia or pseudoachondroplasia due to different mutations in *COMP* gene show flattened, widened, robust femoral necks, decreased inclination of acetabular roofs that are shallow (dysplastic) with pointed lateral margins. The ossification centres of the femoral head are small. The iliac wings appear flared. Adult P1 developed early osteoarthritis in the setting of pre-existing severe deformity of the hip joint.

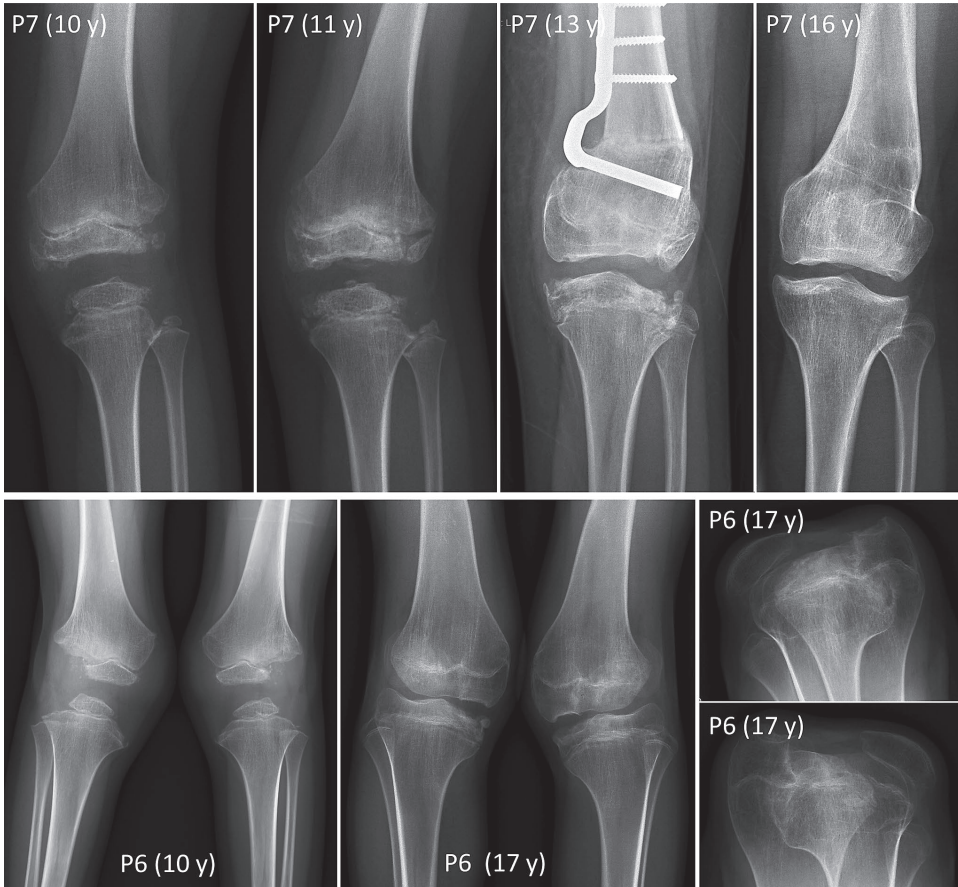


Figure 3 – X-ray survey of the knees (AP and axial views) of P6 and P7 with multiple epiphyseal dysplasia due to mutations *c.1220G>A* in *COMP* gene show progression of bone deformities during growth and ossification. The shortened metaphyses with irregular ossification progressed to deformity of their shape and axial deformity of the proximal tibia and distal femur (surgically corrected in P7), subluxation both in the femoro-tibial and femoro-patellar part of the joint in P7.

were more conspicuous in younger children. The selection of X-ray images of the wrists, pelvis, knees and spine in different age groups is shown in Figures 1–4.

## Discussion

More than 300 mutations in *COMP* gene have already been identified in patients with two different dwarfing conditions including multiple epiphyseal dysplasia (MED/EDM1) and pseudoachondroplasia (PSACH) (Briggs et al., 2014; Chen et al., 2019). Although both disorders still represent individual diseases, an overlap between them was suggested on clinical and radiologic levels (Spranger et al., 2012). The first symptoms usually start in toddlers or preschool children with progression

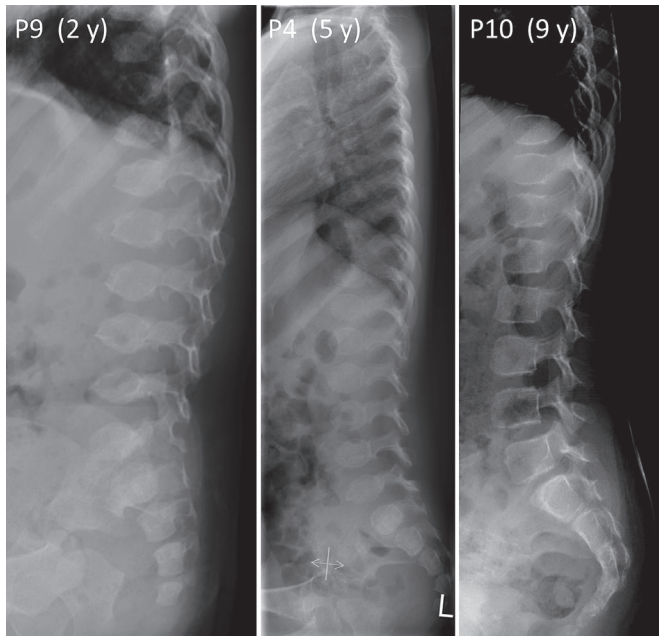


Figure 4 – X-ray survey of the lateral views of the spine of P4, 9 and P10 with multiple epiphyseal dysplasia or pseudoachondroplasia due to different mutations in *COMP* gene show anterior elongation and biconvex configuration of vertebral bodies that are more conspicuous in two younger probands.

of leg deformities and gait and growth disturbances resulting in a very short stature. Although X-ray images may help with the diagnosis of skeletal involvement, a molecular testing is usually necessary for definitive diagnostics (Anthony et al., 2015).

Most patients with PSACH have a mutation in *COMP* gene. The most common is mutation Asp469del, which impedes trafficking of *COMP* and type IX collagen in chondrocytes (Chen et al., 2008). Contrary to PSACH, multiple epiphyseal dysplasia may be except mutation in *COMP* gene also caused by mutations in other genes including *COL9A1* for collagen type IX  $\alpha$ -1, *COL9A2* for collagen type IX  $\alpha$ -2, *COL9A3* for collagen type IX  $\alpha$ -3, and *MATN3* for matrilin-3 resulting in similarly disorganized epiphyseal ossification and destruction of the articular cartilage (Jackson et al., 2004; Hecht et al., 2005; Spranger et al., 2012). In addition, the autosomal recessive variant of MED is caused by a mutation of the sulphate transporter gene *SLC26A2* (Anthony et al., 2015). Therefore, MED belongs to the most genetically heterogeneous disorders between skeletal dysplasias (Unger and Hecht, 2001). Mutations in specific residues or regions of *COMP* are significantly associated with either PSACH or MED but other factors including genetic modifiers are likely to influence the disease severity, which has already been reported in MED caused by *MATN3* mutations (Briggs et al., 2014).

In our patients with heterozygous mutations in *COMP*, the most common variant was c.1220G>A identified in 8 patients with MED from two unrelated families. The variant is present in public Human Gene Mutation Database and according

to the American College of Medical Genetics and Genomics classification is likely pathogenic. It is located in a well-established functional domain (calcium binding type III repeats), where no benign variations are present. The mutation c.1220G>A was previously reported in a sporadic Chinese girl with PSACH (Cao et al., 2011). Her radiographs showed platyspondyly of the spine and anterior fractures of the vertebrae, significant epiphyseal and metaphyseal changes in the joints of the long and short tubular bones, short metacarpal bones and phalanges and small irregular carpal bones compatible with PSACH (Cao et al., 2011). Unlike the Chinese girl, our patients with the same mutation lack a severe impairment of the spine and their phenotypes are more compatible with MED. Our results suggest that not only different mutations, but also the same variant in *COMP* gene may cause either multiple epiphyseal dysplasia or pseudoachondroplasia. This is similar to other amino acid changes in the same protein position as p.Cys407Tyr resulting in MED/EDM1 (Kennedy et al., 2005) or PSACH (Chen et al., 2019). This is supported by experiments described in a *Coll1Tg<sup>COG</sup>* mouse.

The induction of endoplasmic reticulum (ER) stress in the proliferative zone of chondrocytes produced decreased cell proliferation and bone growth and demonstrated the central role of classical ER stress in the MED and PSACH disease process. However, the induced ER stress was not sufficient enough to replicate all of the pathological features of MED and PSACH, suggesting that a combination of more factors including the presence of a defective cartilage extracellular matrix in addition to an increased ER stress is likely to contribute to the pathogenesis of MED and PSACH (Kung et al., 2015; Posey et al., 2019).

Mutations p.Asp376Tyr, p.Asn453Lys and p.Asp446Asn are also located within the type III repeat domain of *COMP*, which is the most common domain for both MED and PSACH. Mutation p.Asp376Tyr was published previously in a patient with PSACH, who accumulated *COMP* and type IX collagen in chondrocytes (Maddox et al., 1997). Mutation p.Asp446Asn has not been described yet, but 3 different substitutions of Asp446 were published in two patients with MED (Zankl et al., 2007; Kim et al., 2011) and one patient with PSACH (Kennedy et al., 2005).

The quality of life is strongly affected in patients with a mutation in *COMP* gene. Treatment is based on the severity of limb deformity, the level of joint destruction, age of the patient and his/her needs. In our group of patients, the most common orthopaedic surgeries during childhood were focused on bowlegs and knock knees. Especially children can benefit greatly from limb realignment procedures. Adults may also have good functional outcomes with joint arthroplasty (Anthony et al., 2015), but discussing the proper timing for the surgery may be of importance. Interestingly, adolescents with idiopathic scoliosis (AIS) have higher *COMP* promoter methylation and lower gene expression, which correlates with young age and high Cobb angle of the main curve. *COMP* promoter methylation may provide prognostic information in predicting the susceptibility and curve progression of AIS (Mao et al., 2018).



## Conclusion

In both multiple epiphyseal dysplasia and pseudoachondroplasia, the skeletal and joint impairment due to mutation in *COMP* start in early childhood. Although the clinical severity is mutation and age dependent, many symptoms represent a continuous phenotypic spectrum between both diseases. Most patients may benefit from orthopaedic surgeries, but the disease still has severe negative impact on their quality of life. Early establishing of the diagnosis is important for genetic counselling and eventual pre-implantation genetic testing.

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# Autologous Hematopoietic Stem Cell Therapy of the Subjects with Systemic Sclerosis: Electromyographic Results of the Masticatory Muscles

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**Abstract:** Musculoskeletal system impairment is a major cause of functional alterations in subjects with systemic sclerosis. Autologous hematopoietic stem cell therapy (AHSCT) may have an important role in the treatment functional of systemic sclerosis patients. The aim of this pilot study was to assess whether AHSCT interferes with the electromyographic activity of the masseter and temporalis muscles of subjects with systemic sclerosis. Before transplantation, seven subjects with systemic sclerosis (mean age [ $\pm$  SD], 40.1  $\pm$  9.6 years) underwent electromyographic analysis of the masseter and temporalis muscles in mandibular tasks at rest, right and left laterality, protrusion and maximum voluntary contraction. Two months after AHSCT, the subjects re-evaluated using the same methods.

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Data were analyzed using the repeated-measure test, with  $p < 0.05$  considered to be statistically significant. Two months after AHSCT, there was reduction in normalized electromyographic activity in the dental clenching in maximal voluntary contraction, with significant differences, for the left temporal muscle ( $p = 0.04$ ). AHSCT in subjects with systemic sclerosis promotes alterations in stomatognathic system function, especially those related to electromyographic activity of masticatory muscles.

## Introduction

Systemic sclerosis is a complex, autoimmune, generalized connective tissue syndrome that presents with chronic fibrotic and inflammatory changes that cause skin tissue thickening and is characterized by microvascular lesions (Sierra-Sepúlveda et al., 2019; Perković et al., 2020). The main clinical signs of systemic sclerosis include limitation of skin movement, muscle pain, weakness, skin thickening, and peripheral vasoconstriction (Mirsaeidi et al., 2019; van Leeuwen et al., 2020).

The incidence of systemic sclerosis has increased over the past decade, although the one-year mortality rate has decreased slightly (Butt et al., 2018). In the United States, the prevalence for this uncommon condition is of approximately 20 persons per million in the general population (Bergamasco et al., 2019).

The disorder frequently involves the orofacial tissues (Crincoli et al., 2016), along with muscular atrophy that contributes to bone resorption compromising the temporomandibular joint, atrophy of the skin and face, hardening and loss of elasticity of the oral mucosa, and difficulties with swallowing and chewing (Iordache et al., 2019; Mendonça et al., 2019).

Systemic sclerosis is a multidisciplinary disease, as it involves many organs and systems, leading to a wide range of symptoms and limitations. The treatment of this autoimmune disease remains a major challenge to the medical field and, as such, new therapeutic options are continually being sought (Sobolewski et al., 2019).

In the past 20 years, autologous hematopoietic stem cell transplantation (AHSCT) has emerged as a treatment option for patients with severe and progressive systemic sclerosis. Randomized studies have shown that transplantation is superior to conventional immunosuppressive treatment, promoting longer survival, better disease control and improved quality of life (van Laar et al., 2014; Sullivan et al., 2018).

Functional deterioration is associated with AHSCT (Wiskemann and Huber, 2008) and shortly after transplantation, patients are advised to reduce the amount of functional activity (Dimeo et al., 2003). However, this inactivity can have the opposite effect, impairing muscle function, so therapy is a promising treatment that restores muscle function, even in a short period of time (Plowman et al., 2014).

Thus, the aim of this pilot study was to evaluate electromyographic activity of the masticatory muscles, before and two months after selected subjects with systemic sclerosis underwent AHSCT. The null hypothesis was that autologous stem cell transplantation does not influence the electromyographic activity of the masticatory muscles after two months of treatment in subjects with systemic sclerosis.

## Material and Methods

### *Study design*

This study was approved by the Research Ethics Committee (protocol No. 94010718.4.00000.5419), based on Resolution 466/2012 of the Brazilian National Health. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (University of Sao Paulo, Brazil) and with the Helsinki Declaration of 1975, as revised in 2008. Informed consent was obtained from all subjects who participated in the study.

Sample size calculation was based on a study using G\* Power version 3.1.9.2 software (Franz Faul, Kiel University, Kiel, Germany), a priori considering  $\alpha=0.05$ , an effect size of 1.41, and a test power of 87% for the main result of EMG (electromyography) activity in subjects with systemic sclerosis (Hausmanowa-Petrusewicz et al., 1982). The minimum sample size obtained was seven.

In this prospective, longitudinal study, recruitment and selection of subjects were performed by open invitation of systemic sclerosis patients treated at the University Hospital of the Ribeirão Preto Medical School, Brazil. All subjects had a confirmed diagnosis of systemic sclerosis according to the 2013 American College of Rheumatology/European League Against Rheumatism criteria (Johnson, 2015) and fulfilled previously published (Burt et al., 2013) indications for AHSCT. All subjects were assessed by a questionnaire that determined the presence or absence of temporomandibular dysfunction according to the Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD). Dental evaluation was performed using anamnesis and a clinical form.

At the beginning of this study, 20 subjects with natural dentition were evaluated for the presence of first permanent molars, and for normal occlusion without temporomandibular dysfunction. Subjects for whom AHSCT was denied due to exclusion criteria for transplantation (Burt et al., 2011) were ineligible for the present study. Furthermore, subjects with ulceration(s), open wounds or skin hypersensitivity, cognitive impairment, neurological and/or systemic (decompensated) disorders, edentulous, use of full or removable prosthesis, periodontal disease, parafunctional habits, mandibular torus and/or palatine, and those using muscle relaxants that could interfere with neuromuscular physiology were excluded.

Ten systemic sclerosis patients were included in the present study. However, three subjects died between the initial analysis and the two months after therapy time point. Therefore, seven subjects participated in the study (mean age [ $\pm$  SD],  $40.1 \pm 9.6$  years). They initially underwent electromyographic analysis of the masseter and temporalis muscles. Two months after treatment, these seven subjects with systemic sclerosis were re-evaluated with the same methods.

Evaluation of electromyographic activity was performed by a single trained professional. Intra-examiner calibration was performed for all analyses in this study.

### *Hematopoietic stem cell collection*

Transplant procedure has been previously published (Burt et al., 2011). Briefly, stem cells were mobilized from the bone marrow to the peripheral blood with intravenous cyclophosphamide, followed by granulocyte colony growth factor (G-CSF) injections (van Laar et al., 2014). Stem cell collection was performed by leukapheresis; unselected cells were cryopreserved (Blank et al., 2016).

Pre-transplant preparation included high doses of cyclophosphamide and antithymocyte globulin, aiming to ablate the immune system and eliminate autoreactive cells. Then, the hematopoietic and immune systems were restored through infusion of the previously harvested autologous hematopoietic stem cells.

### *Electromyographic analysis*

Electromyographic activity of the masseter and temporalis muscles, reflected by the patterns of muscle fiber activation, was recorded using a wireless system (Trigno, Delsys Inc., Boston, MA, USA).

The sensors were positioned on the subjects by the same operator trained and trained according to the Surface ElectroMyoGraphy for the Non-Invasive Assessment of Muscles (SENIAM) recommendations (Hermens et al., 2000). Dental clenching manoeuvres in maximal voluntary contraction were performed to establish the optimal collection points for electromyographic signals of the masseter and temporalis muscles (Cecílio et al., 2010).

Electromyographic signal data, in microvolts/s, were calculated using the square mean root for mandibular tasks. The skin was sanitized with alcohol to decrease impedance and the sensors were fixed after a few minutes of this procedure (Di Palma et al., 2017).

Electromyographic signal recording adhered to the following protocol for mandibular tasks: rest (4 s); right (4 s) and left (4 s); laterality, protrusion (4 s); and dental clenching in maximal voluntary contraction with (4 s) and without (4 s) inert material.

The inert material consisted of a 12-folded paraffin sheet (Parafilm M, Pechinery Plastic Packaging, Batavia, IL, USA) (18×17×4 mm, weight 245 mg) and inserted between the occlusal faces of the first permanent molars, on both the right and left sides of the dental arch (Siéssere et al., 2009).

### *Statistical analysis*

In the analysis of the results, the data showed normal distribution (Shapiro-Wilk normality test:  $p < 0.05$ ). The electromyographic values for the masseter and temporalis muscles were normalized with respect to the electromyographic amplitude values obtained from each muscle during a maximal voluntary clench on Parafilm M. Statistical analysis of normalized electromyographic data was performed using SPSS version 22.0 (IBM Corporation, Armonk, NY, USA) for Windows



(Microsoft Corporation, Redmond, WA, USA). The data were analyzed using a repeated measure test. Statistical significance was set at the level of  $p < 0.05$ .

## Results

Table 1 shows the normalized electromyographic data from the masseter and temporalis muscles at rest and under mandible postural conditions before and after AHST in systemic sclerosis subjects. Lower normalized electromyographic activity was observed in the dental clenching in maximal voluntary contraction of the left temporal muscle ( $p=0.04$ ), when compared to baseline (pre-therapy) measurements.

**Table 1 – Normalized electromyographic data and mean  $\pm$  standard error of the right masseter (RM), left masseter (LM), right temporal (RT) and left temporal (LT) muscles in the mandibular tasks of subjects with systemic sclerosis before (GI) and after (GII) autologous hematopoietic stem cell therapy (AHST)**

Variable	GI	GII	p-value
Total sample size	07	07	NA
<b>Variables – muscle</b>			
Rest – RM	0.25 $\pm$ 0.10	0.19 $\pm$ 0.10	NS
Rest – LM	0.29 $\pm$ 0.12	0.20 $\pm$ 0.07	NS
Rest – RT	0.47 $\pm$ 0.16	0.18 $\pm$ 0.03	NS
Rest – LT	0.29 $\pm$ 0.06	0.26 $\pm$ 0.08	NS
Right laterality – RM	0.59 $\pm$ 0.23	0.29 $\pm$ 0.11	NS
Right laterality – LM	0.53 $\pm$ 0.19	0.32 $\pm$ 0.10	NS
Right laterality – RT	0.46 $\pm$ 0.18	0.18 $\pm$ 0.04	NS
Right laterality – LT	0.30 $\pm$ 0.10	0.19 $\pm$ 0.06	NS
Left laterality – RM	0.48 $\pm$ 0.18	0.20 $\pm$ 0.08	NS
Left laterality – LM	0.41 $\pm$ 0.17	0.19 $\pm$ 0.04	NS
Left laterality – RT	0.65 $\pm$ 0.24	0.26 $\pm$ 0.11	NS
Left laterality – LT	0.24 $\pm$ 0.06	0.21 $\pm$ 0.09	NS
Protrusion – RM	0.62 $\pm$ 0.26	0.14 $\pm$ 0.03	NS
Protrusion – LM	0.29 $\pm$ 0.14	0.23 $\pm$ 0.08	NS
Protrusion – RT	0.35 $\pm$ 0.16	0.14 $\pm$ 0.02	NS
Protrusion – LT	0.31 $\pm$ 0.08	0.23 $\pm$ 0.07	NS
Dental clenching – RM	1.02 $\pm$ 0.14	0.82 $\pm$ 0.09	NS
Dental clenching – LM	1.05 $\pm$ 0.13	0.85 $\pm$ 0.11	NS
Dental clenching – RT	0.93 $\pm$ 0.07	0.80 $\pm$ 0.06	NS
Dental clenching – LT	1.02 $\pm$ 0.16	0.73 $\pm$ 0.08	0.04*

\*significant difference, repeated-measure test (i.e.  $p < 0.05$ ); NA – not applicable (paired samples); NS – not significant

## Discussion

The null hypothesis of this study was rejected by the positive influence of therapy according to electromyographic activity of the masseter and temporalis muscles in subjects with systemic sclerosis.

AHSCT in patients with systemic sclerosis induces the production of new cells from the immune and hematopoietic systems, for the purpose of eliminating autoreactivity and, as consequence, recovering the patient's general health (Lee et al., 2016). Skeletal muscles are involved in this autoimmune disease (Costa-Pereira et al., 2019), however, little is known about how masticatory muscles behave in the context of systemic sclerosis.

The involvement of striated skeletal muscles in subjects with systemic sclerosis is a consequence of ischemic, inflammatory, and sclerotic processes due to oxygenation and insufficient nutrition of the muscular tissue (Giudice et al., 2018). After AHSCT, there is a noteworthy increase in the quantity of blood vessels, which in turn improves peripheral and visceral blood circulation, and decreases in the activity of inflammatory processes (Khodayari et al., 2019). These factors may be related to reduced myoelectric activity due to increased blood flow in muscle tissue, promoting lower intramuscular pressures and, in consequence, functional improvement (McNeil et al., 2015).

Two months after transplantation, a reduction in normalized electromyographic activity of the temporal muscle in the dental clenching in maximal voluntary contraction was observed. Importantly, the most active muscle in this neuroanatomical movement is the masseter, which has a very rich arterial supply (Hwang et al., 2001). It is well known in the medical field that subjects treated with AHSCT exhibit more active patterns in organizing vascular architecture, with microcapillary regeneration (Miniati et al., 2009). We therefore believe that these functional outcomes may be related to an enriched balance and reduced electromyographic activity of the masticatory muscles due to microvascular tissue remodelling, increased tissue oxygenation, and improved muscle performance.

Another hypothesis to explain the reduction in electromyographic activity in the postural condition of dental clenching in maximum voluntary contraction would be that the muscle involvement through electromyographic evaluation of subjects with systemic sclerosis may be related to the formation of mandibular osteolysis that promotes imbalance(s) in electromyographic activity (Maiti et al., 2018). The transplantation could promote osteometabolic regeneration of the mandible, with reduction in bone ischemia and in muscle tissue pressure over the mandible angle (Heino and Hentunen, 2008), thus leading to functional improvement. In this study, however, the presence of mandibular osteocytes was not evaluated.

However, we can mention that in systemic sclerosis there is an increase in choline concentration due to the proliferation of inflammatory cells and thereby, an increase in myoelectric activity (Sener et al., 2019). What would explain the reduction in electromyographic activity? With AHSCT, there is a reduction in the number of

inflammatory cells, thus improving the functional status of subjects with autoimmune disease (Menasché, 2018).

This pilot study had limitations. First the number of patients is very low. Second the method of SENIAM may be burdened with a larger measurement error. Third the study does not present a comparative set of healthy individuals to determine any standard results. Fourth limitation would be the evaluation period of the stomatognathic system after autologous stem cell transplantation (i.e. two months), may have been insufficient. A longer period of evaluation after therapy would have probably resulted in significant data.

## Conclusion

The results suggest that patients with systemic sclerosis treated with AHSCT may present improvements in electromyographic activity of the masticatory muscles, especially observed in the dental clenching in maximal voluntary contraction of the left temporal muscle. Therefore, this study opens up great horizons in many areas of research to better understand the functional behavior of the stomatognathic system in subjects with systemic sclerosis undergoing AHSCT.

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# Serum Galectin-3 Levels Are Unlikely to Be a Useful Predictive Marker for Early-onset Preeclampsia Development

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**Abstract:** Galectin-3 (gal-3) is lectin which is presumed to interact with extracellular matrix proteins and cell surface glycoproteins in normal and pathophysiological conditions. The expression of gal-3 at the fetal-maternal interface partially overlaps that of gal-1, suggesting that an interplay between them might be important for hypertensive disorders in pregnancy like preeclampsia. The aim of our study was to test the hypothesis whether galectin-3 could be used as a predictive marker for early-onset preeclampsia development. 32 patients with early-onset preeclampsia were examined, mean age  $28.8 \pm 5.5$ ; and 22 age matched normal pregnancies mean age  $28.5 \pm 6.0$ . The enzyme-linked immunosorbent assay (ELISA) was used for measuring serum galectin-3 levels. There were no significant differences between serum levels of galectin-3 in sera of preeclampsia patients compared to normal pregnant women –  $14.1 \pm 4.77$  vs.  $15.7 \pm 5.95$  ng/ml ( $p > 0.05$ ). Serum galectin-3 levels correlated with maternal age ( $r = 0.33$ ;  $p = 0.03$ ) and BMI (body mass index) ( $r = 0.52$ ;  $p = 0.01$ ). Our data suggest that determination of serum galectin-3 levels may not be a useful method for prediction of early-onset preeclampsia. Studies should be aimed to other categories of biomarkers.

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## Introduction

Preeclampsia (PE) has not been fully studied yet. It is a major cause of maternal and perinatal morbidity and mortality (Gallo et al., 2016). PE and gestational hypertension (GH) are associated with preterm delivery, intrauterine fetal death and increased maternal morbidity/mortality rate (Villar et al., 2006). Currently many biomarkers for early detection and prognosis of preeclampsia are investigated. The most widely used markers in clinical practice are: placental growth factor (PLGF) (Tsiakkas et al., 2015; Sung et al., 2017) and serum pregnancy-associated plasma protein-A (PAPP-A) (Kalousová et al., 2014; Patil et al., 2014). Unfortunately, their sensitivity and specificity is low yet. There is growing evidence that galectins are involved in pathophysiology of preeclampsia. However, preeclampsia does not always clinically present when remodelling has failed to occur (Karumanchi and Granger, 2016). Biomarkers of early preeclampsia detection are critical for risk stratification and testing therapies (Ferguson et al., 2017). Therefore, there is an urgent need for high quality, novel research in PE biomarkers, so that the best predictive strategy can be identified in order to improve the management of women in high risk.

Galectin-3 is an intra- and extracellular lectin. It is presumed to have an interaction with extracellular matrix proteins and cell surface glycoproteins. Currently, the physiological functions of this protein are in intensive studies. Ochieng et al. (1998) have established that recombinant human galectin-3 is a novel substrate for matrix metalloproteinases-2 and -9 (MMP-2 and MMP-9). Results from their study suggest that cleavage of galectin-3 by metalloproteinases alters the lectin's carbohydrate domain recognition. This process helps the tight glycoconjugates' binding. In result galectin molecules self-association is reduced.

“Galectin-3 is observed in fibroblasts, chondrocytes, osteoblasts, osteoclasts, keratinocytes, Schwann cells and gastric mucosa. It is also found in endothelial cells in a number of tissues, and in immune cells such as neutrophils, eosinophils, basophils, mast cells, Langerhans cells and dendritic cells (cell types reviewed in). It plays a role in adipocyte proliferation, and obese mice have more galectin-3 in adipocytes than lean subjects have. Galectin-3 can be both antiapoptotic and proapoptotic inductor. Galectin-3's potential therapeutic use is in large focusing on inhibition, because gal-3 promotes cancer and metastasis, and inhibition would hence have a great potential for therapeutic anti-cancer treatment” (Brinchmann et al., 2018).

An interesting difference between galectin-1 and galectin-3 is that galectin-3 is considered a pro-inflammatory signal targeting various innate immune aspects (i.e. macrophages, mast cells, and neutrophils) to promote their activation, degranulation, and cytokine production (Alves et al., 2010).

Consistent with the confirmed antagonistic effects of galectin-1 and galectin-3 on T-cell responses *in vitro*, ovine placental galectin-3 has been observed to promote T-cell proliferation and activation (Iglesias et al., 1998). In mice, uterine galectin-3 expression is selectively upregulated during early stages of pregnancy and localizes

to the luminal and glandular epithelia and the primary decidual zone, whereas at later stages this lectin is predominantly expressed in the placenta (Phillips et al., 1996). This distribution, collectively with the lowered implantation rates discovered upon tissue-specific galectin-3 knock-down in the uterus (Yang et al., 2012), suggests the pivotal function of this lectin during the embryo-maternal cross-talk driving implantation. Galectin-3 well-shows a comparable spatio-temporal distribution throughout pregnancy, being upregulated in the endometrium at the peri-implantation duration and detected in the placental villous cytotrophoblasts; and extravillous trophoblasts lineages as gestation progresses (Blois and Barrientos, 2014).

Despite the appreciable characterization in several species, the physiological features of galectin-3 expression at the fetal-maternal interface are unexplored yet. To this moment there is small number of studies investigating galectins and especially galectin-3 as a marker predicting the development of preeclampsia.

The available data from galectin-3 studies in aspect of its involvement in preeclampsia development so far are:

- 1) Elevations in serum galectin-3 levels with increments in insulin resistance-related parameters and lipid profiles reflect the possible contribution of gal-3 to the harmful effects of IR (insulin resistance) and dyslipidemia levels on women with PE;
- 2) Galectin-3 may be both an initiating factor in the pathophysiology of preterm prelabor rupture of membranes, a marker in the prediction, and a target of preventing strategies of preterm prelabor rupture of membranes.

Therefore, we decided to test the hypothesis whether galectin-3 could be used as a predictive marker for early-onset preeclampsia development.

## Material and Methods

### *Patient population*

All patients were residing in the vicinity of the University Hospital Pleven. Subjects' sera were taken from October 2019 to February 2020. All the procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional or regional) and with the Helsinki Declaration of 1975, as revised in 2000. Approval of local Ethics Committee was obtained and informed consent from adult research participants was obtained too. The study group consisted of 32 patients with preeclampsia, mean age  $28.8 \pm 5.5$ ; and 22 age matched normal pregnancies mean age  $28.5 \pm 6.0$  (Table 1).

### *Study inclusion criteria*

- Pregnant women with clinical symptoms and laboratory criteria for early-onset preeclampsia (preeclampsia between 20 and 34 gestational week)
- Maintaining a current diet and exercise during the study
- Lack of metabolic syndrome and/or diabetes mellitus

**Table 1 – Clinical data of women with preeclampsia and normal pregnant women**

	Preeclampsia	Normal pregnant women
Maternal age	28.8 ± 5.5	28.5 ± 6.0
BMI ≥ 25	23/32*	4/22
Gravida	2 (2)**	3 (2)**
Parity	1 (2)**	1 (2)**
SBP (mm Hg)	138 ± 17*	116 ± 14
DBP (mm Hg)	81 ± 10*	68 ± 8
Past history of preeclampsia	1/32	8/22*
Family history of hypertension	11	19*
Chronic hypertension	1/32	10/22*
Abnormal UAD	18/32*	2/22
Smoker	6/32	2/22
Galectin-3 (ng/ml)	14.1 ± 4.77	15.7 ± 5.95
Number	(n=32)	(n=22)

BMI – body mass index; SBP – systolic blood pressure; DBP – diastolic blood pressure; UAD – uterine artery Doppler; data are shown as the mean ± SD (standard deviation); \*p<0.05; \*\*data are expressed as median (interquartile range)

- Parameters of hypertension
- Presence/absence of risk factors
- Signed informed consent approved by the Ethics Committee at the University Hospital “G. Stranski” and the Medical University of Pleven

#### *Study exclusion criteria:*

- Diabetes mellitus
- Kidney disease
- Cardiovascular disease
- Signs of chorioamnionitis
- Presence of a fetus with chromosomal abnormality

#### *Diagnostic criteria for preeclampsia*

Preeclampsia is defined by hypertension in pregnancy\* and coexistence of one or more of the following new-onset conditions\*\*:

*\*Definition and classification of hypertension in pregnancy according to 2018 ESC guidelines for the management of cardiovascular diseases during pregnancy:*

The definition of hypertension in pregnancy is based only on office (or in-hospital) BP values ([systolic BP [SBP] ≥ 140 mm Hg and/or DBP ≥ 90 mm Hg) and distinguishes mildly (140–159/90–109 mm Hg) or severely (≥ 160/110 mm Hg) elevated BP, in contrast to the grades used by the joint ESC/ESH Hypertension Guidelines.

*Definition of early-onset and late onset preeclampsia according ISSHP  
(International Society for the study of Hypertension in Pregnancy)*

Early-onset preeclampsia (EOP) is defined before 34 weeks' gestation, and late-onset preeclampsia (LOP) is defined after 34 weeks or later.

*\*\*Proteinuria*

Spot urine protein/creatinine > 30 mg/mmol (0.3 mg/mg) or > 300 mg/day or at least 1 g/l ("2+") on dipstick testing.

*\*\*Other maternal organ dysfunctions*

- 1) Renal insufficiency (creatinine > 90 µmol/l; 1.02 mg/dl)
- 2) Liver involvement (doubling of serum transaminases and/or severe right upper quadrant pain)
- 3) Neurological complications (eclampsia, altered mental status, blindness, stroke, or more commonly hyperreflexia when accompanied by clonus and severe headaches when accompanied by hyperreflexia and persistent visual scotomata)
- 4) Hematological complications (platelet count < 150,000/dl, DIC, and hemolysis)

*\*\*Uteroplacental dysfunction*

Fetal growth restriction

*Blood pressure measurement*

Arterial blood pressure was measured using a standard aneroid sphygmomanometer, to the nearest 2 mm Hg, in the dominant arm after at least 10-min rest in supine position. Blood pressure measuring was performed by "Riester" blood pressure measuring tool – type –Precisa® N; Ø 64 mm aluminium, single-tube, cotton hook cuff, adult, No. 1362-104.

*Electrocardiography*

Electrocardiography (ECG) was performed for LVH (left ventricular hypertrophy) assessment (Sokolow-Lyon index > 35 mm, or R in a VL ≥ 11 mm; Cornell voltage duration product > 2,440 mm/ms, or Cornell voltage > 28 mm in men or > 20 mm in women). ECG was performed by 12-lead ECG machine "Fukuda" – type – FX 8322.

*Doppler of the uterine artery*

Flow velocity waveforms of the uterine artery were performed by ultrasound apparatus using an AB 2–7 MHz convex abdominal probe (GE Voluson E10, GE Medical Systems, Zipf, Austria). Patients were evaluated once in the semirecumbent position by a single operator after bed rest for 5 minutes. Three consecutive waveforms were obtained in the Doppler study. The mean pulsatility index (PI) was

calculated. An abnormal Doppler of uterine artery result was diagnosed as a mean PI > the 95<sup>th</sup> percentile for each gestational age.

#### *Enzyme-linked immunosorbent assay (ELISA)*

It was used for measuring galectin-3 levels. Gal-3 levels were measured in serum samples using enzyme-linked immunosorbent assay (Galectin-3 Quantikine ELISA Kit R and D Systems, Minneapolis, MN, RD-DGAL30) according to the manufacturer's instructions.

#### *Statistical analyses*

The research data was processed with the computer programs Excel (Microsoft Corporation, Redmond, WA) and Statgraphics Plus (Manugistics, Rockville, MD) for Windows. All results were described in tables, graphs, numerical values (mean  $\pm$  SD, share indicators and correlations). The level of significance was determined as ( $p < 0.05$ ). The one-way analysis of variance with F-test in the ANOVA table was used for means comparison. K-W (Kruskal-Wallis) test was performed in cases with different from normal distribution. Median (M) was used in K-W test, together with first and third quartile Q1 and Q3 (twenty-fifth and seventy-fifth percentile P25 and 75P). The Pearson test was used for assessing of correlations. Multivariate regression analysis was also performed.

## **Results**

There were no significant differences between serum levels of galectin-3 in preeclampsia women compared to normal pregnant women –  $14.1 \pm 4.77$  vs.  $15.7 \pm 5.95$  ng/ml ( $p > 0.05$ ) (Figure 1). Serum galectin-3 levels correlated with maternal age ( $r = 0.33$ ;  $p = 0.03$ ) and BMI (body mass index) ( $r = 0.52$ ;  $p = 0.01$ ).

## **Discussion**

Preeclampsia is a common pregnancy complication. It is associated with high maternal morbidity and mortality rates and intrauterine foetal growth restriction.

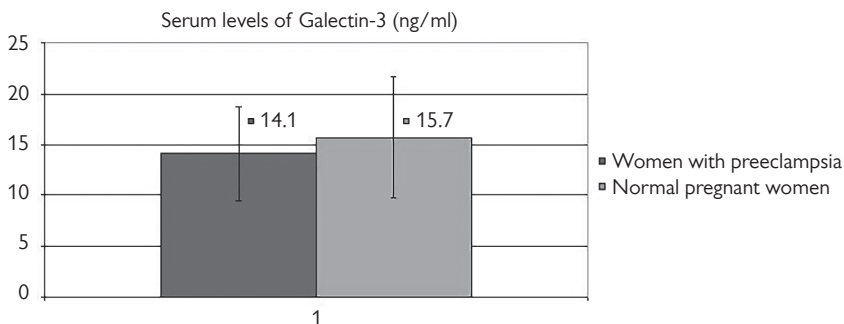


Figure 1 – Serum levels of galectin-3 in sera of women with preeclampsia and in normal pregnancies.

This condition is characterised by high blood pressure and significant amount of protein in urine. Early detection of preeclampsia is very important for patient's risk stratification and an adequate management. It is extremely important for clinicians to perform risk assessment of developing preeclampsia. "The ASPRE trial (Combined Multimarker Screening and Randomized Patient Treatment with Aspirin for Evidence-based Preeclampsia Prevention): aspirin from 12 weeks concluded that treatment with low-dose aspirin in women at high risk for preterm PE reduces substantially the incidence of this disease". That is why the Fetal Medicine Foundation recommends preeclampsia pharmacologic prevention by admitting a prophylactic low-dose acetylsalicylic acid per os (150 mg/day) in high risk women (Rolnik et al., 2017).

Hirashima et al. (2018) evaluated whether the serum level of galectin-1 (gal-1) at 18–24 and 27–31 weeks of gestation is a risk factor for predicting the later occurrence of not only preeclampsia (PE) but also gestational hypertension (GH). They measured serum levels of soluble fms-like tyrosine kinase 1 (sFlt-1), placental growth factor (PlGF), and gal-1 using an enzyme-linked immunosorbent assay in normal pregnant women, women with a later onset of GH, and women with a later onset of PE at 18–24 and 27–31 weeks, respectively. Authors also determined gal-1 in 33 women with GH and women with PE after the onset. The levels of gal-1 after the onset of GH, late-onset PE (onset at  $\geq 34$  weeks), and early-onset PE (onset at  $< 34$  weeks) were significantly higher than those in normal pregnant women at 27–31 weeks. Authors' data show that, the serum level of gal-1 is a novel risk factor for both GH and PE, specifically its expression at a low level in the second trimester and a high level after onset.

Taha et al. (2020) study aimed to determine the correlations amongst serum apelin and galectin-3 levels and insulin resistance (IR) in women with PE. Authors made conclusion, that elevations in serum galectin-3 levels with increments in IR-related parameters and lipid profiles reflect the possible contribution of galectin-3 to the harmful effects of IR and dyslipidemia levels on women with PE.

Kaya et al. (2020) investigated maternal serum galectin-1 and galectin-3 levels in pregnancies complicated with preterm prelabor rupture of membranes (PPROM) and compared it with pregnancies delivered at term. Patients complicated with PPRM had significantly higher levels of galectin-1 and galectin-3 than the control group. Maternal serum galectin-3 levels were found significantly negatively correlated with the gestational age at delivery and birth weight. Maternal serum galectin-1 and galectin-3 levels were significantly higher in pregnancies complicated with PPRM. Galectin-1 and galectin-3, with their regulatory effects in key biological processes, may be both an initiating factor in the pathophysiology of PPRM, a marker in the prediction, and a target of preventing strategies of PPRM.

These studies (Hirashima et al., 2018; Kaya et al., 2020; Taha et al., 2020) have reported that galectins are involved in pathophysiology of preeclampsia. In our study we tested the hypothesis whether galectin-3 could be used as a biomarker predicting



early-onset preeclampsia development. Our results did not support the hypothesis. We report, using the latest study cohort to date, that serum galectin-3 levels are unlikely to be a useful marker of early-onset preeclampsia development.

Possible explanations could be:

- The expression of galectin-3 at the fetal-maternal interface partially overlaps that of galectin-1, suggesting that an interplay between these lectins might be important for developmental processes and immune modulation during early pregnancy.
- It is known that galectin-3 can have both antiapoptotic and proapoptotic properties depending on its association with intracellular or extracellular binding partners. Additionally, this lectin appears to play an important role in the modulation of adaptive immunity, as it has been shown that galectin-3 can either promote or inhibit T-cell apoptosis.
- Unlike galectin-1, galectin-3 is considered a pro-inflammatory signal targeting diverse innate immune components (i.e. macrophages, mast cells, and neutrophils). Probably pregnancy promote their activation, degranulation, and cytokine production in a different way, which need further investigations.
- Galectin-3 interacts mainly with matrix metalloproteinase-2 and matrix metalloproteinase-9, but not with matrix metalloproteinase-1, which is known to be involved in the breakdown of components of extracellular matrix. Specifically, matrix metalloproteinase-1 breaks down the interstitial collagens, types I, II, and III.
- Galectin-3 expression is up regulated in the very early stages of preeclampsia, even before 20 weeks of gestation. The inclusion criteria for our study was pregnant women with early-onset preeclampsia (preeclampsia between 20 and 34 gestational week).

## Conclusion

Serum galectin-3 levels are unlikely to be a useful marker of early-onset preeclampsia development. Studies should be aimed to other categories of biomarkers.

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# Rare Tumours of the Testis: Twelve Years of Experience

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**Key words:** Testis tumours – Rare tumours – Gonadal tumours

**Abstract:** Rare tumours of the testis includes a wide variety of tumours. We aim to present clinical and histological characteristics of our patients with rare tumours of the testis. The medical records of 33 patients who were treated and followed-up for testicular rare tumours in our center between 2007 and 2020 were retrospectively reviewed. Of all the 243 testicular tumours, 222 cases (91.4%) were germ cell tumours and 21 cases (8.6%) were non-germ cell tumours. Thirty-three rare tumours of the testis including rare germ cell tumours and non-germ cell tumours were detected. The mean age of the patients at diagnosis was 34 years (range 18–68 years). The histological types of rare testicular tumours were as follows: teratoma 4.5% (n=11), sex-cord stromal tumours 4.5% (n=11), paratesticular tumours 3.2% (n=8), and the others [lymphoma 0.4% (n=1), mesothelioma 0.4% (n=1) and choriocarcinoma 0.4% (n=1)]. The median duration of follow-up was 32 months (range 1 to 256 months). None of the patients with non-metastatic disease stage developed recurrence after having received appropriate therapy. Metastatic disease was documented in 9 cases at the time of diagnosis (five patients with teratomas, two patients with Leydig cell tumour, one patient with choriocarcinoma and rhabdomyosarcoma). The most common subtypes of testicular rare tumours in our center was teratoma and sex-cord stromal tumours. Because of testicular rare tumours have different biological features and different clinical outcomes, the management of each tumour requires a different approach.

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## Introduction

Testicular tumours constitute less than 1% of all male cancers and occur especially between the ages of 15 and 35 years. Testicular germ cell tumour is the most common type of testicular malignancies (approximately 95% of all testicular tumours). Testicular germ cell tumours include a heterogeneous group of neoplasms such as seminoma, yolk sac tumours, embryonal carcinoma, mixed germ cell tumours, teratoma, and choriocarcinoma (Williamson et al., 2017). The remaining (5%) involves non-germ cell tumours such as sex-cord stromal tumours, testicular lymphomas, and paratesticular tumours [sarcoma (liposarcoma, leiomyosarcoma, rhabdomyosarcoma)] (Williamson et al., 2017; Mooney and Kao, 2018).

The clinical features of more common germ cell tumours such as seminoma, yolk sac tumours, embryonal carcinoma, mixed germ cell tumours are well known. The optimal treatment approaches have been standardized, and this has led to excellent treatment outcomes (Howitt and Berney, 2015). However, we know little about rare testicular cancers including teratoma, choriocarcinoma, and non-germ cell tumours.

There are no studies involving large series of patients with rare tumours of the testis that would guide management, and available data is limited to the case reports and case series. Therefore, it is important that the centers share their own experiences.

The main objective of the present study was to analyse the cases documented in our department to determine the relative frequency of testicular rare tumours in adults and to report clinical and histological characteristics, treatment and outcomes of these tumours.

## Material and Methods

We performed a retrospective analysis of patients with testicular tumours in the archived records between 2007 and 2020. Clinical characteristics, histopathological findings, laboratory values and treatment data of the patients were recorded from the hospital records and computer data system. A total of 243 cases with testicular tumour have been reviewed. All the cases of germ cell and non-germ cell tumours were separated according to the histological subtype. Also, the files of patients diagnosed with teratoma and choriocarcinoma, which are rare from germ cell tumours, were separated.

Thirty-three patients with rare tumours of the testis including rare germ cell tumours and non-germ cell tumours were identified (Table 1). The parameters evaluated were as follows: age at diagnosis, clinical characteristics (presentation at diagnosis), preoperative serum tumour marker [alpha fetoprotein (AFP), human chorionic gonadotropin (HCG)] levels, histopathological findings, stage, treatment methods, and treatment outcome.

## Results

Of all the 243 testicular tumours, 222 cases (91.4%) were germ cell tumours and 21 cases (8.6%) were non-germ cell tumours. Of all germ-cell tumours, 36.6% (n=89)

**Table 1 – The general features of our patients**

Histopathology	Mean age (years)	No (%) (total =243)	Right /left	Stage				Radical orchiectomy		Treatment		Median follow-up (months)	Relaps (n)	Outcome + response status		
				I	II	III	IV	(+)	(-)	surveillance	KT/RT				BSC	
Rare germ cell tumours	23	12 (4.9%)														
Teratoma	23	11 (4.5%)	5/6	2	4	5	–	11	0	0	0	9 (BEP/EP)	2	106 (1–256)	0	1 complete response, 3 partial response
Choriocarcinoma	23	1 (0.4%)	0/1	0	0	1	–	1	0	0	0	1 BEP	0	1	0	exitus after 1 cycle BEP
Non-germ cell tumours		21 (8.6%)														
Sex-cord stromal tumours	40	11 (4.5%)												33 (1–87)		
Leydig cell tumours	38.5	8 (3.2%)	7/1	6	0	2	–	8	0	6	1	1 (BEP/EP)	1	28 (1–87)	0	1 stable disease
Sertoli cell tumours	67	1 (0.4%)	0/1	1	0	0	–	1	0	1	0	0	0	33	0	0
Sertoli-Leydig cell tumours	46	1 (0.4%)	0/1	1	0	0	–	1	0	1	0	0	0	44	0	0
Granulosa cell tumours	44	1 (0.4%)	1/0	1	0	0	–	1	0	0	0	1 (P+C)	0	2	0	0
Paratesticular tumours	32	8 (3.2%)														
Adenomatoid tumour	32	1 (0.4%)	1/0	1	0	0	0	1	0	1	0	0	0	2		
Sarcomas		5 (2%)												24 (4–95)		
Liposarcoma	56	1 (0.4%)	1/0	0	1	0	0	1	0	0	0	1 RT	0	95	0	0
Rhabdomyosarcoma	19.5	2 (0.8%)	1/1	1	0	0	1	2	0	0	0	2 (VAC/IE)	0	55 (24–86)	0	1 progression
Fibrosarcoma	18	1 (0.4%)	0/1	0	1	0	0	1	0	1	0	0	0	4	0	0
Fibromyxoid sarcoma	55	1 (0.4%)	0/1	1	0	0	0	1	0	1	0	0	0	8	0	0
Leiomyoma	39	2 (0.8%)	2/0	2	0	0	0	1	0	2	0	0	0	25 (4–46)	0	0
Others		2 (0.8%)														
Lymphoma	68	1 (0.4%)	0/1	0	0	1	0	1	0	0	0	1 (R-CHOP)	0	5	0	0
Mesothelioma	68	1 (0.4%)	0/1	1	0	0	0	1	0	0	0	1 RT	0	10	0	0
Total	34	33 (13.6%)	18/15	17	6	9	1	33	0	13 (39.4%)	17 (51.5%)	3 (9.1)	3 (9.1)	32 (1–256)	0	0

BSC – best supportive care; KT – radiotherapy; RT – radiotherapy; BEP – bleomycin, etoposide, cisplatin; P+C – paclitaxel plus carboplatin; VAC – vincristine, doxorubicin, cyclophosphamide; EP – etoposide, cisplatin; R-CHOP – rituximab, cyclophosphamide, doxorubicin, vincristine, prednisolone; IE – ifosfamide, etoposide

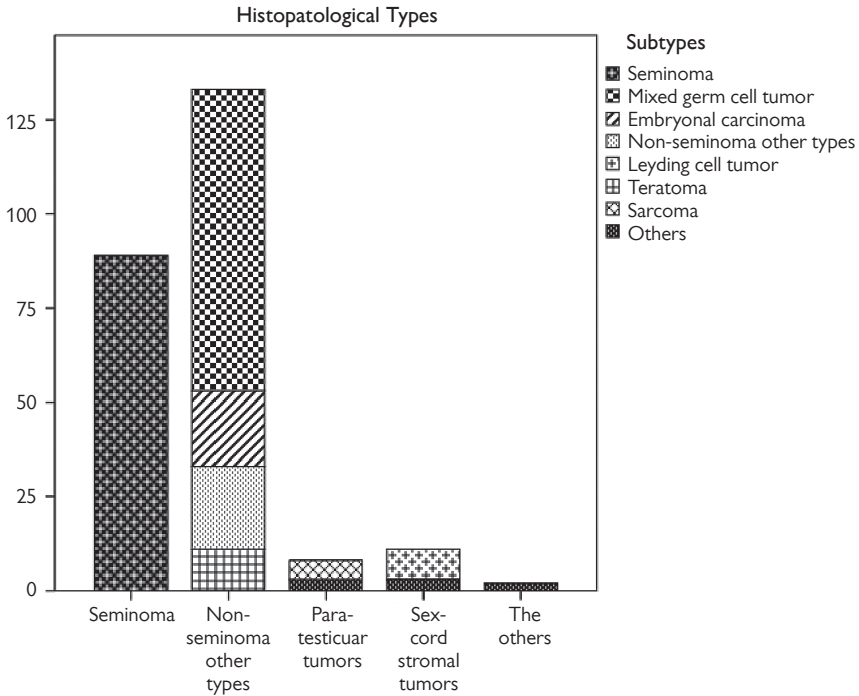


Figure 1 – Histopatological types and subtypes of testicular tumours.

were seminoma, 32.9% (n=80) were mixed germ cell tumour, 8.6% (n=21) were yolk sac tumour, 8.4% (n=20) were embryonal carcinoma, and 4.9% (n=12) were rare germ cell tumours (teratoma and choriocarcinoma).

The clinical data of the 33 patients who were diagnosed with rare tumour of the testis (non-germ cell tumours and rare germ cell tumours) are presented in Table 1. The mean age at presentation was 34 years (range 18–68 years). The most common presentation (>95%) was a painless scrotal mass or swelling. Two patients had a history of trauma, and one of the patients had a history of cryptorchidism. No family history was reported. None of the patients had endocrine symptoms such as gynecomasty. All 33 testicular tumours were treated with radical orchiectomy. The median size of primary tumour mass in the testicle was 43 mm (range 5–170 mm). The diameters of most tumours were more than 4 cm. The median duration of follow-up in all patients was 32 months (range 1–256 months). With regard to the laterality, the tumour was located in the right in 18 patients (54.5%) and in the left in 15 patients (45.5%).

The histological subtypes of rare testicular tumours were as follows: teratoma, 4.5% (n=11, 11/243); sex-cord stromal tumours, 4.5% (n=11); paratesticular tumours, 3.2% (n=8); and others [lymphoma 0.4% (n=1), mesothelioma 0.4% (n=1)



and choriocarcinoma 0.4% (n=1)]. The rates of histopathological subtypes are shown in detail in Figure 1.

The mean age of the patients with testicular neoplasms of different histology were 23, 40 and 32 years in patients with teratoma, sex-cord stromal tumours and paratesticular tumours, respectively. In histopathological examination, the presence of necrosis, mitotic activity (>5) and angiolymphatic invasion were observed in stage III Leydig cell tumours. Also, necrosis and mitotic activity (>5) were reported in liposarcoma and rhabdomyosarcoma.

Based on our experience of more than 12 years on the rare tumours of the testis, we found that 11 cases (4.5%) had sex-cord stromal tumours [eight cases with Leydig cell (six cases at stage I and 2 case at stage III (M1)), one case with Sertoli (at stage I), one case with Sertoli-Leydig cell (at stage I) and one case with granulosa (at stage I)]. The patient diagnosed with high risk stage I granulosa cell tumour underwent retroperitoneal lymph node dissection (RPLND) and received adjuvant carboplatin plus paclitaxel. Of these 2 patients, one underwent chemotherapy (BEP/EP) (BEP – bleomycin, etoposide, cisplatin) (EP – etoposide, cisplatin) and had stable disease at follow-up; the remaining one patient who did not receive chemotherapy and was followed with best supportive care, showed progression.

In total, 11 out of 33 (4.5%) patients were diagnosed with teratoma. Testicular teratomas comprised 4.9% (11/222) of patients with germ cell tumours. Five patients with teratoma presented with stage III disease (2 cases had stage I and four cases had stage II disease) and 1 patient with choriocarcinoma had stage III disease (M1). One patient with testicular lymphoma presented with stage III disease. One patient with mesothelioma had stage I disease. Of patients with paratesticular tumours, two had rhabdomyosarcoma (one had stage I and the other had stage IV), two patients had leiomyoma, one patient had stage I fibromyxoid sarcoma, one patient had stage II liposarcoma, one patients had stage II fibrosarcoma, and one patient had adenomatoid tumour. Of the 33 patients, two patients had benign tumours, including one patient with leiomyoma (0.8%; 2/243) and one patient with adenomatoid tumour (0.4%).

Serum  $\beta$ -HCG ( $\beta$ -human chorionic gonadotropin) was elevated in three patients with stage III teratoma and one patient with choriocarcinoma. In addition, LDH (lactate dehydrogenase) was elevated in patients with stage III teratoma, lymphoma, stage IV rhabdomyosarcoma and choriocarcinoma.

Adjuvant chemotherapy was administered to eight patients with early stage rare testicular tumour according to the histological subtype [BEP regimen in 6 patients diagnosed with teratoma, VAC/IE (VAC – vincristine, doxorubicin, cyclophosphamide; IE – ifosfamide, etoposide) regimen in 1 patient with rhabdomyosarcoma, carboplatin plus paclitaxel regimen in 1 patient with granulosa cell tumour], and 2 patients received only adjuvant radiotherapy (2 cases diagnosed with mesothelioma and liposarcoma). R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, prednisolone) was given to the patient with a testicular

lymphoma. Thirteen patients were actively followed-up after radical orchiectomy since they had low-risk early stage disease. None of the patients with non-metastatic disease developed recurrence after having received appropriate therapy and being follow-up [median duration of follow-up, 32 months (range 1–256)] without evidence of disease.

Metastatic disease was documented in 9 of these cases at the time of diagnosis. Three out of nine patients with metastatic disease (two cases with teratoma, one case with Leydig cell tumour) received no palliative therapy and were followed-up with best supportive care because of poor performance status. The remaining six patients received chemotherapy pursuant to the histological subtype (BEP in patients with teratomas and choriocarcinoma, carboplatin and paclitaxel in patients with Leydig cell tumour, VAC/IE in patients with alveolar rhabdomyosarcoma). One patient with choriocarcinoma died of metastatic disease after the first cycle of the BEP regimen. Complete response was observed in one of the patients with a metastatic teratoma, and partial response was achieved in two patients. A patient with rhabdomyosarcoma had progressive disease at the end of 3 months after having received the VAC/IE regimen. A patient with a metastatic Leydig cell tumour showed stable response to the BEP regimen, and the patient died of metastatic disease (median duration of follow-up was 28 months). Twenty-four patients survived (median duration of follow-up, 32 months), whereas 9 died of active disease. The deaths occurred in patients with Leydig cell tumour, teratoma, choriocarcinoma, rhabdomyosarcoma, mesothelioma and lymphoma.

## Discussion

Rare tumours of the testis include a wide variety of tumours such as non-germ cell tumours and rare germ cell tumours (teratoma and choriocarcinoma), each presenting with different histopathological and clinical characteristics. Therefore, many different treatment approaches require for some histological types of rare testis tumours.

In general, a painless testicular mass or swelling, occasionally accompanied by a hydrocele is probably the typical presenting symptom of the rare tumours of the testis, as it is within in testicular germ cell tumours (Howitt and Berney, 2015). In our study, the most common presenting symptom (>95%) was a painless scrotal mass or swelling. We also observed that there was a mild predominance of right-sided teratomas (right/left, 54.5%/45.5%). As in germ cell tumours, there is probably no clear evidence for a predisposition of one side due to limited data (Dieckmann et al., 2018).

Testis tumours are mainly observed between the ages of 20 and 45 years. However, rare tumours of the testis have a different age distribution. Malignant Leydig cell tumours, testicular lymphomas, and paratesticular sarcomas are rare testicular tumours observed in individuals older than 60 years of age (Gigantino et al., 2013). But in our study, the mean age at presentation was 34 years (range

18–68 years). The mean age of the patients with testicular neoplasms of different histological subtypes was 23, 40 and 32 years in the groups of teratoma, sex-cord stromal tumours and paratesticular tumours, respectively.

Serum AFP (alpha-fetoprotein) and  $\beta$ -HCG are important tumour markers in detecting primary, recurrent, or metastatic disease as well as in follow-up of germ cell tumours. Teratomas and choriocarcinoma are rare tumours of the testis that can lead to increased serum AFP or  $\beta$ -HCG concentrations. But tumour markers are negative in all sex-cord stromal tumours and thus routine measurement during follow-up is not required (Azizi et al., 2020). Three patients with metastatic teratomas and one patient with choriocarcinoma had elevated  $\beta$ -HCG levels before and after surgery. In addition, LDH elevation was detected in all patients with stage III (M1) teratoma, lymphoma, stage IV rhabdomyosarcoma and choriocarcinoma. The remaining patients had normal serum markers.

In adult patients with testicular tumours, the specific histological diagnosis is based on the resected specimen. In daily practice, as malignant tumours of the testis are far more common than benign tumours in adulthood, radical orchiectomy has become standard approach among the urologic surgeons (Gigantino et al., 2013). In our study, of 243 patients with testicular tumours, 222 (91.4%) had germ cell tumours and 21 (8.6%) had non-germ cell tumours. Thirty-three patients with rare tumours of the testis including rare germ cell tumours and non-germ cell tumours were identified. We showed that in our center, most of the rare tumours of the testis were malignant. The most common histological subtype of the rare testicular tumours was teratoma (4.5%) and sex-cord stromal tumours (4.5%). Our results are consistent with the rate of testicular cancer types reported in previous studies (David et al., 2017; Osbun et al., 2017).

The 5-year survival rate of patients with testicular germ cell tumours is more than 90% (Williamson et al., 2017). But 5-year survival rate for rare testicular tumours and the prognosis of patients with these tumours differ across the tumour subtypes. The incidence and frequency of each tumour subtype varies considerably. Also, therapeutic modalities including surgery, radiation, and chemotherapy varies according to the histological type and stage (Azizi et al., 2020).

Pure teratoma of the testis is uncommon in adults, accounting for fewer than 5% of all testicular germ cell tumours. Teratomas may contain mature or immature elements and present as a component of mixed testicular germ cell tumours or rarely present as pure neoplasms (Cheng et al., 2017). Also, testicular teratomas can occasionally show transformation into somatic-type malignancies (Mikuz and Colecchia, 2012). In the latest World Health Organization (WHO) classification, pure testicular teratoma is divided into two main forms based in part on the patient's age: prepubertal and postpubertal types. Postpubertal-type teratoma is clinically aggressive, while pure prepubertal-type teratoma has an indolent clinical course (Cheng et al., 2017). The standard treatments are radical surgery and cisplatin-based chemotherapy and/or radiotherapy based on tumour stage for germ

cell tumours. Also, retroperitoneal lymphadenectomy is recommended in case of residual lesion after chemotherapy. In a retrospective study of 543 cases diagnosed with germ cell tumour, only 14 (2.5%) had pure teratomas. Adult patients with malignant testicular teratomas consisted of 79% of all patients with pure teratoma cases in this series. Patients with testicular teratomas consisted of 4.9% (11/222) of patients with germ cell tumours in our study that involved 243 patients. Five cases with teratoma presented with advanced disease [stage III (M1)]. All of the cases had malignant teratoma. Three of the cases received curative BEP regimen. Complete response was achieved in one patient with metastatic teratoma, and partial response was achieved in two patients.

Sex-cord stromal tumours constitute the great majority of non-germ cell tumours of the testis, accounting for only 4% of all testicular tumours (Mooney and Kao, 2018). Leydig cell tumours and Sertoli cell tumours are the most common subtypes, accounting for 1–2% and 0.5% of all testis tumours, respectively (Conkey et al., 2005). Granulosa cell tumour is a rare type of sex-cord stromal tumours that are represented by juvenile and adult subtypes. Endocrine symptoms [gynecomastia (>15%), rarely Cushing syndrome, precocious puberty] are frequently observed in Leydig and granulosa cell tumours. Germ cell tumours and sex-cord stromal tumours are seen more commonly in young patients and are mostly benign, and approximately 5% to 10% have malignant behaviour (Conkey et al., 2005; Dieckmann et al., 2019). Patients with stage I disease and malignant characteristics had a lower 5-year survival rate (77–94%) compared to those with stage I germ cell tumour (98%). Also, five-year survival of patients with stage I Sertoli cell tumours is significantly lower than in those with Leydig cell tumour. Patients with metastatic disease have poor survival and these tumours are usually refractory to chemotherapy and radiotherapy (Banerji et al., 2016).

There is a lack of consensus on the optimal treatment approach in cases with testicular rare tumours. Most information regarding treatment and survival outcomes of malignant sex-cord stromal tumours come from small retrospective studies. Radical orchiectomy alone is generally curative for early stage sex-cord stromal tumours and benign tumours. The other options include retroperitoneal lymph node dissection (RPLND), radiation, chemotherapy or a combination therapy (Banerji et al., 2016; Azizi et al., 2020). Chemotherapy regimens have included bleomycin, etoposide and cisplatin, or doxorubicin and cisplatin (platinum regimens). In the absence of metastatic disease on imaging studies, active surveillance may be also a reasonable option. Primary RPLND with or without adjuvant chemotherapy may be performed in selected patients with sex-cord stromal tumours, especially in high-risk patients. For patients with clinical stage I sex-cord stromal tumours with two or more malignant characteristics, primary RPLND is a recommended treatment strategy (Azizi et al., 2020).

In a study involving a total of 48 men with testicular sex-cord stromal tumours [Leydig cell (28), Sertoli cell (13), granulosa cell (2) and unclassified (5) tumours],

Silberstein et al. (2014) reported 34 patients with no malignant characteristics at partial or radical orchiectomy who were successfully followed-up without primary RPLND and experienced no tumour recurrence during a limited follow-up duration (median 14.5 months). In our study, we found that 11 cases (4.5%) had sex-cord stromal tumours [eight patients had Leydig cell (3.2%), one patient had Sertoli (0.4%), one patient had Sertoli-Leydig (0.4%), and one patient had granulosa (0.4%)]. None of the patients had a history of gynecomasty. Six patients presented with stage I Leydig tumour at the time of initial diagnosis, and two patients had stage III disease (M1). Of these 2 patients, one underwent chemotherapy (BEP) and had stable disease during the follow-up; the remaining patient who did not receive chemotherapy and was followed with best supportive care showed progression. Adjuvant therapy was not administered to six patients with stage I Leydig cell tumour and 3 patients with the other types of sex-cord stromal tumours and not directed to RPLND since they did not have malignant characteristics. These patients remained disease-free during a follow-up period of 33 months after radical orchiectomy. But a patient who was diagnosed with high-risk stage I granulosa cell tumour underwent RPLND and received adjuvant carboplatin plus paclitaxel. Poor prognostic characteristics such as large size (5 cm or greater), necrosis, nuclear atypia, vascular invasion, high mitotic rate (more than 5 per 10 high-power fields) and age > 50 years increase the likelihood of malignancy in a testicular mass (Bremmer et al., 2014; Mooney and Kao, 2018).

Paratesticular tumours originate from the paratesticular soft tissue region (spermatic cord, epididymis and tunica vaginalis). These tumours are rare, accounting for fewer than 5% of all testicular masses. The majority of all paratesticular masses are benign and include lipomas, adenomatoid tumours, fibroma, hemangioma, and leiomyomas. Adenomatoid tumours are the most common paratesticular tumours occurring predominantly in patients aged 30 to 50 years as an asymptomatic and slow-growing mass. Surgical excision is curative because it is benign (Amin, 2005; Mooney and Kao, 2018). In our study, the benign tumours were leiomyoma and adenomatoid tumour. There were two patients with leiomyoma and one patient with adenomatoid tumour. The rate of benign lesions may be underestimated, because benign tumours are less likely to be referred to the department of oncology.

Paratesticular sarcomas occur rarely in adults, with a peak incidence between 55 and 65 years of age (Keenan et al., 2019). Approximately 30% of paratesticular tumours have malignant histology. Malignant paratesticular sarcomas include liposarcomas, rhabdosarcomas, leiomyosarcomas and malignant fibrous histiocytomas (Keenan et al., 2019). Liposarcoma is the most common type of paratesticular sarcomas. The majority are well-differentiated sarcomas. Recurrence can occur locally in well-differentiated sarcomas but have no metastatic potential in the absence of dedifferentiation. Dedifferentiated sarcomas have the potential to metastasize (Mooney and Kao, 2018; Keenan et al., 2019). The main prognostic

factors are tumour size, grade and nodal or distant metastasis (Gigantino et al., 2013). Overall five-year cancer-free survival ranges from approximately 60% to 80% (Khoubehi et al., 2002). In our study, paratesticular sarcomas were as follows; two cases with rhabdomyosarcoma at stage I and at stage IV (0.8%), one case with fibromyxoid sarcoma at stage I (0.4%), one case with liposarcoma at stage II (0.4%), one fibrosarcoma at stage II (0.4%). Rhabdomyosarcoma is a rare tumour, mostly seen in the first two decades of life unlike the other paratesticular sarcomas. Rhabdomyosarcomas tend to be more aggressive than paratesticular tumours. Rhabdomyosarcoma was reported to have a high incidence of lymph nodes metastasis and distant metastasis.

There is no consensus on the optimal treatment approach in patients with paratesticular sarcoma. However, aggressive surgical resection should be performed in patients with paratesticular sarcomas to ensure surgical margin negativity (Keenan et al., 2019). The role of retroperitoneal lymph node dissection is uncertain, especially in liposarcoma and leiomyosarcoma. Retroperitoneal lymph node dissection is recommended in some cases of intermediate-grade or high-grade lymph node involvement on imaging studies of patients with liposarcoma and leiomyosarcoma, and in all patients over 10 years of age as part of staging for rhabdomyosarcomas (Khoubehi et al., 2002; Dangle et al., 2016). Some experts advocate the use of chemotherapy in metastatic disease. Adjuvant chemotherapy in paratesticular sarcomas has not been evaluated in previous studies. The local relapse rate after orchiectomy is 25–37% for paratesticular tumours. Adjuvant radiotherapy reduces locoregional recurrence (Khoubehi et al., 2002). Fagundes et al. (1996) have reported no local recurrence in patients receiving adjuvant radiation after orchiectomy, compared with those treated with orchiectomy alone (37%). In our study, a patient diagnosed with stage II liposarcoma received only adjuvant radiotherapy. This patient is followed in recurrence-free remission.

The current treatment has been standardized for rhabdomyosarcoma unlike the other paratesticular sarcomas. Multimodal approach including radical orchidectomy and/or RPLND, multiagent chemotherapy (VAC/IE regimen) and/or radiation is required (Dangle et al., 2016). The patients with stage I rhabdomyosarcoma received VAC/IE regimen without RPLND. The patient at stage I is followed free of recurrence. The patient at stage IV had progressive disease at the end of 3 months after VAC/IE regimen and died of metastatic disease at twenty-four months of the disease.

In our study, each of the other cases, including those with choriocarcinoma, mesothelioma and lymphoma of rare germ cell tumours, make up 0.4% of the total number of cases. Choriocarcinoma of the testis is extremely rare in the testis but can be seen as a component of a mixed germ cell tumour. Most patients are between 10 and 30 years of age. Serum  $\beta$ -HCG levels are markedly elevated (Cheng et al., 2017). The prognosis of choriocarcinoma is poor without treatment. But there is a chance of cure with combination chemotherapy even in patients with a



metastatic disease (Howitt and Berney, 2015). Our patient with choriocarcinoma died of metastatic disease after 1 cycle BEP regimen.

Malignant mesothelioma of the tunica vaginalis testis is an extremely rare tumour, representing 0.3% to 5% of all malignant mesotheliomas (Chekol and Sun, 2012). It occurs most commonly in men in the sixth or seventh decades of life. Exposure to asbestos is the only known risk factor (Amin, 2005). The prognosis for patients with malignant mesothelioma is poor, with a median survival of 23 months (Chekol and Sun, 2012). Aggressive therapy with radical orchiectomy and chemotherapy for advanced or recurrent disease remains the mainstay of therapy, usually with poor response. Our patient had asbestos exposure. After orchiectomy, the patient received adjuvant radiotherapy due to risk factors (>5 cm tumour size, rete testis and lymphovascular invasion).

Testicular lymphomas are rare, accounting for approximately 1–2% of all non-Hodgkin lymphomas and 5% of testicular malignancies (Gigantino et al., 2013). It is the most common type of testicular cancer in men older than 60 years of age. The majority of testicular lymphomas (80–98%) are histologically diffuse large B-cell lymphoma (Twa et al., 2018). The patients with testicular lymphomas have the heightened risk of central nervous system (CNS) relapse. The prognosis for this lymphomas is poor. In a review, overall survival at 5 and 10 years for stage I patients was 58 and 29%, and for stage II patients, it was 46 and 29%, respectively (Zucca et al., 2003). But patients with stage IV disease have a relapse rate greater than 90% and a 5-year survival of 20–25% (Gigantino et al., 2013). The standard treatment for testicular DLBCL (diffuse large B-cell lymphoma) generally includes orchiectomy and anthracycline, rituximab containing chemotherapy regimens, with or without locoregional radiotherapy and prophylactic scrotal radiotherapy in addition to central nervous system-directed prophylaxis (Cheah et al., 2014). There was a 68-year-old patient with testicular lymphoma in our study. The histopathology was follicular lymphoma. The patient was found to have stage III disease on imaging studies. The patient started a R-CHOP chemotherapy regimen. After five months, follow-up data is not available. There are case reports in literature on follicular lymphoma of the testis.

The limitations of this study include small sample size and retrospective study design. Despite these limitations, we presented our own data on rare tumours of the testis.

In conclusion, the most common subtypes of testicular rare tumours in our center were teratoma and sex-cord stromal tumours. Testicular rare tumours in adults have various tumour types, because of rare occurrence and limited clinical data of these tumours, optimal treatment strategies are challenging. Because testicular rare tumours have different biological features and different clinical outcomes, the management of each tumour requires a different approach. Clinical experience in testicular rare tumours is based on a few small case series and predominantly on single case reports. Therefore, it is important to make treatment decisions of patients with testis rare tumours in tumour boards.

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# Type-I Cryoglobulinaemia Associated to Monoclonal Gammopathy of Undetermined Significance

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**Key words:** Cryoglobulinaemia – Monoclonal gammopathy of undetermined significance – Neuropathy – Vasculitis

**Abstract:** Cryoglobulins are immunoglobulins that undergo reversible precipitation at cold temperatures. Monoclonal type-I cryoglobulinaemia is the least frequent and is associated to hematological diseases such as multiple myeloma, Waldenström’s macroglobulinaemia, chronic lymphocytic leukaemia and lymphoma. We describe the case of a 60-year-old female patient, who suffered from burning pain in her feet for ten months before her admission. The patient presented intermittent distal cyanosis that progressed to digital ischaemia. She also reported paresthesia in her hands, difficulty in writing, and a 26-kg-weight loss. At the physical examination, it was identified livedo reticularis, palpable purpura, and painful ecchymotic lesions in her calves and feet. Moreover, peripheral pulses were palpable and symmetrical. It was observed an atrophy of the right first dorsal interosseous and both extensor digitorum brevis, as well as a distal bilateral apalesthesia and allodynia. Both Achilles reflexes were absent. Laboratory tests revealed anemia, high erythrocytation rate and C-reactive protein. Serum protein electrophoresis showed a monoclonal IgG-Kappa gammopathy. The results also evidenced the presence of Bence-Jones proteinuria. The bone marrow biopsy revealed less than 10% of plasma cells, and skin biopsy informed leukocytoclastic vasculitis. The patient was treated with high-dose intravenous steroids and cyclophosphamide. The treatment showed that the skin lesions had improved, pain disappeared and motor deficit stopped its progression.

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## Introduction

Cryoglobulins are immunoglobulins that precipitate with cold and can be monoclonal or polyclonal. Type-I cryoglobulinaemia is the least common form of presentation. It is monoclonal and is usually associated with lymphoproliferative diseases such as multiple myeloma, Waldenström macroglobulinaemia, chronic lymphocytic leukemia and lymphoma (Ramos-Casals et al., 2012; Ramos et al., 2017). It follows the description of the case of an adult patient who developed ischemia, digital and peripheral vasculitis neuropathy secondary to type-I cryoglobulinaemia related to monoclonal gammopathy of undetermined significance (MGUS). The established immunosuppressive therapy achieved a clear improvement of symptoms.

## Case report

A 60-year-old woman who, 10 months before her admission, started feeling burning pain in both soles and intermittent distal cyanosis in both lower extremities, which were exacerbated by standing and exposition to cold, was admitted. In the last month it evolved with a persistent blueish coloration in the 3<sup>rd</sup> to 5<sup>th</sup> toes of the right foot, with necrotic lesions. She reported paresthesia in both hands and difficulty in writing and fine mobility in the right hand for the last month. She noticed a twenty-six-kilogram weight loss for the last 6 months, related to a hypocaloric diet. She denied fever, arthralgia and colour changes in the hands or face. Nor did she refer any other relevant symptoms in her past personal and familiar history.

On physical examination, it was found a presence of livedo reticularis, palpable purpura and ecchymotic lesions, painful at palpation, in both the anterior and medial legs (Figure 1). The third right toe showed bluish colour and distal necrosis. Peripheral pulses were present. No edema was found.



Figure 1 – Distal necrosis of the third toe; purpuric lesions in the left lower limb.

The neuromuscular examination showed loss of strength in the right interosseous muscles (MRC scale 3/5); the strength was normal at the remaining muscles; hyporeflexia in the right upper limb and both lower limbs, as well as bilateral indifferent plantar reflexes. She presented atrophy of the first right dorsal interosseum and both extensor digitorum brevis muscles; she described allodynia in legs and feet.

Stein Weinstein's monofilament test was positive on both soles of the feet. The rest of the physical examination, including the fundus and the capillaroscopy, was normal.

The electromyogram determined the existence of a sensory-motor axonal neuropathy in the lower limbs, with denervation, associated with the dysfunction of A-delta fibers (abnormal silent cutaneous period). In the upper extremities, a multiple axonal mononeuritis was evident.

Imaging studies of the chest, abdomen and pelvis showed no pathological findings, except for signs of hepatic steatosis. Arterial and venous Doppler ultrasound of both lower extremities was normal.

The presence of osteolytic lesions in the skull or long bones was not evident. Renal function and urinary sediment were normal. Total body positron emission tomography showed no abnormalities.

In the admission laboratory Hb (hemoglobin) 10.4 g/dl (reference value (RV) = 12.0–16.0) compatible with anemia of chronic disorders; erythro sedimentation > 120 mm/h (VR < 30) and C-reactive protein 3.83 mg/dl (RV < 0.3 mg/dl). The rest of the laboratory showed no relevant findings.

Antibodies against HIV, hepatitis B and C were negative. VDRL (venereal disease research laboratory test) was not reactive.

The C4 fraction of the complement decreased: 4 mg/dl (RV = 16.0–47.0) and the C3 fraction was normal.

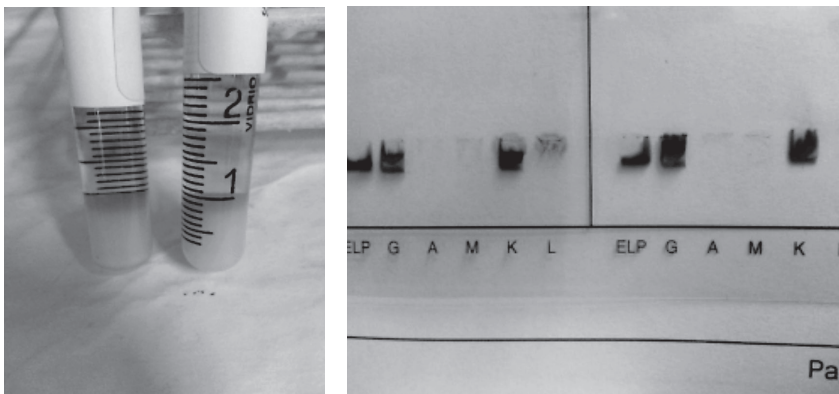


Figure 2 – Left: cryoglobulin precipitation from serum; right: immunofixation, monoclonal IgG-Kappa type-I cryoglobulin.

The electrophoretic proteinogram showed a monoclonal component of 1.9 g/dl in the gamma fraction. Serum electro immunofixation determined an IgG-Kappa monoclonal gammopathy. Proteinogram with immunofixation of the urine showed Bence Jones proteinuria of the Kappa type. The quantification of serum immunoglobulins by immunoturbidimetry resulted in: IgG 1,530 mg/dl (RV = 658–1,837), IgA 45 mg/dl (RV = 71–360) and IgM 87 mg/dl (RV = 40–263).

The determination of serum cryoglobulins was positive: cryocrit 16% (RV < 0.5). Immunochemical typing of purified cryoglobulin (immunofixation): monoclonal IgG-Kappa type-I cryoglobulin (Figure 2). The viscosity of the serum was normal. The PCR for hepatitis C virus in the cryocrit sample was negative.

The determination of rheumatoid factor, antinuclear antibodies (FAN/AAN-IFI-HEp-2, linear immunoassay), Anti DNA, Anti-Sm, Anti-U1snRNP, Anti-Scl-70, Anti-SSA/Ro (52), Anti-SSA/Ro (60), Anti-SSB/LA, Anti-Cemp-B, anti-Histones, Anti-Rib-P and ANCA were negative.

The myositis profile of the antibodies was negative: method: linear immunoassay. Substrate: highly purified or recombinant antigens (Anti-Jo-1, Anti PM/Scl-100, Anti PL-12, Anti Mi-2, Anti Ku (p70/80), Anti SRP, Anti PL-7) antibodies.

The determination of the antiganglioside-IgG/IgM-ELISA antibodies (Anti GM1, anti-Asialo GM1, anti-GM2, anti-GD1a, anti-GD1b and anti-GQ1b) was negative. The anti-MAG-IgM-ELISA antibody (myelin-associated glycoprotein) was negative.

A bone marrow biopsy puncture was performed: it showed abnormalities of the plasma cells compatible with monoclonal gammopathy of uncertain importance. The skin biopsy revealed leukocytoclastic vasculitis.

The diagnosis was a type-I cryoglobulinaemia vasculitis associated with a monoclonal gammopathy of undetermined origin (MGUS): immunosuppressive treatment was initiated with high-dose corticosteroids and intra-venous cyclophosphamide. The skin lesions improved, burning pain in the lower extremities subsided and the progression of motor involvement stopped.

## Discussion

The patient began her disease with dermatological and neurological manifestations that worsened with cold, due to a type-I cryoglobulinaemia, associated with an IgG-Kappa MGUS. This entity corresponds to 22% of all patients with cryoglobulinaemia, followed by multiple myeloma and Waldenström macroglobulinaemia. Cutaneous involvement due to vascular purpura and peripheral neuropathy are the most frequent manifestations (Sidana et al., 2017).

The clinical manifestations are due to deposits of immune complexes in the vascular wall of the skin, joints, the peripheral nervous system or the kidney. Capillaries, arterioles and cryocrit are primarily involved, but small and medium-sized arteries can also be affected (Damoiseaux and Cohen Tervaert, 2014).

The most frequent cutaneous sign (in 80–90% of patients) is palpable purpura, which always begins in the lower extremities; the trunk and upper extremities are



less involved. Raynaud's syndrome and acrocyanosis can occur (Cacoub et al., 2015). In our patient, all of them were dermatological manifestations, in addition to ulceration and necrosis in the third toe of the left foot, which are the most frequent signs in this type of cryoglobulinaemia (Kolopp-Sarda and Miossec, 2018). Histopathological lesion of the skin was compatible with leukocytoclastic vasculitis: this manifestation is not frequent in type-I cryoglobulinaemia, albeit it has been reported (Echeverría et al., 2011).

Neuropathy associated with cryoglobulinaemia is found in 7–17% of symptomatic patients. It consists of an axonal damage secondary to necrotizing vasculitis or intraneural infiltration of cryoglobulins. Most vasculitis neuropathies are multiple mononeuritis or symmetric motor-sensory neuropathy. In some patients, the presence of anti-GM1 or anti-sulfatide antibodies (Thomas et al., 1992) may be detected. Painful neuropathies secondary to cryoglobulinaemia might also be caused by other contributing factors, such as the increased expression of proinflammatory cytokine genes or nerve growth factor (NGF).

In summary, there is a reduction in the density of intraepidermal nerve fibers in this painful neuropathy (Blaes, 2015; Hsu et al., 2017). The patient described had an axonal neuropathy with concomitant involvement of the small fibers in the lower extremities; and in the upper extremities, a multiple mononeuritis of the axonal type. Anti-GM1 antibodies were negative and the presence of anti-sulfatide antibodies could not be determined.

Our patient was diagnosed with IgG-Kappa MGUS, without anti-MAG antibodies. These antibodies are more frequently associated with IgM MGUS and cause a paraproteinemic demyelinating neuropathy, by interfering with the signalling between the axon and the Schwann cell: a reduction in the phosphorylation of neurofilaments and a reduction in the axonal caliber is generated, thus reducing the efficiency of nerve (Ramos et al., 2015; Talamo et al., 2015). MGUS is considered a premalignant condition, as it is associated with lymphoproliferative disorders such as multiple myeloma, Waldenström macroglobulinaemia, chronic lymphocytic leukaemia and lymphoma (Kagaya and Takahashi, 2005).

Type-I cryoglobulins lack rheumatoid factor (FR) activity and do not readily activate complement. In this case report, complement C4 was greatly diminished and C3 was normal, typical of mixed cryoglobulinaemia.

The association of corticosteroids and cyclophosphamide led to the improvement of the skin and the peripheral neurological syndrome, with a decrease in painful symptoms. In the largest study of type-I cryoglobulinaemia associated with MGUS, most patients received this treatment scheme. Other treatments performed in that study were rituximab or only corticosteroids. The improvement was 57% in those patients with MGUS IgG and 86% in those with IgM MGUS (Sidana et al., 2017).

In conclusion, we present the case of a woman with type-I cryoglobulinaemia associated with IgG-Kappa MGUS, which manifested mainly with peripheral neuropathy and dermatological lesions, without renal complications.

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