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Qualitative and Quantitative Characteristics of the Passive Compartment Underneath the Myocardial Bridge Comprising the Blood Vessel Adventitia and the Perivascular Connective Tissue

Características Cualitativas y Cuantitativas del Compartimento Pasivo Debajo del Puente Miocárdico que Comprende la Adventicia del Vaso Sanguíneo y el Tejido Conectivo Perivascular

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SUMMARY: Myocardial bridges are inborn anomalies frequently found in authopsies. Although tipically clinically silent, they are occasionally associated with severe clinical manifestations, e.g. myocardial ischemia or even sudden death. The pathophysiology and risk factors for these manifestations have not yet been completely elucidated. The connective tissue underneath the bridge has been considered as one of the factors the symptoms depend on. Thus, the aim of this research was to determine the histological characteristics of the connective tissue lying underneath the myocardial bridge and to contribute to a better understanding of the protective effects this passive compartment might have in prevention of severe clinical manifestations of myocardial bridging. The study was carried out on twenty hearts with myocardial bridges. Length of the bridge was determined using a precise electronic caliper. Sections of the myocardial bridges with the underlying connective tissue were obtained and prepared for qualitative and quantitative analysis. The connective tissue underneath the bridges was composed of adipose tissue and loose connective tissue in different ratios. The tissue underneath thin bridges was predominantly composed of adipose tissue, while loose connective tissue was the dominant component under thick bridges. The myocardial bridges had an average thickness of 0,98 ± 0.44 mm and an average length of 15,25±5,65 mm. We found a strong positive correlation between the myocardial bridge thickness and length (r = 0,860, p = 0,0001). The thickness of the passive connective tissue compartment under the myocardial bridges was 0,58±0,22 mm, and there was no correlation between this parameter and the myocardial bridge thickness (r = -0,011; p = 0,963). In the clinical evaluation of patients with these anomalies it is necessary to take into account independently the myocardial bridge thickness and length on one side and the thickness of the connective tissue lying underneath it on the other.

KEY WORDS: Myocardial bridge; Perivascular connective tissue; Tunica adventitia.

INTRODUCTION

A myocardial bridge (MB) is a congenital anomaly in which a major epicardial artery passes one part of its route through the myocardium (Alegria *et al*., 2005; Hostiuc *et al*., 2017; Javadzadegan *et al*., 2019). It is mostly found over the left anterior descending artery (LAD) (Corban *et al*., 2014; Li *et al*., 2016; Torii *et al*., 2018). The 'tunneled' segment of the artery is compressed during each systole, and this results in diastolic blood flow changes/disturbances (Kim *et al*., 2009; Ural *et al*., 2009; Li *et al*., 2016; Javadzadegan *et al*., 2019). According to different autopsy series, myocardial bridging occurs relatively often (in up to 40-80 % of cadavers) (Möhlenkamp *et al*., 2002; Alegria *et al*., 2005; Kim *et al*., 2009). In contrast to that, the systolic narrowing observed by coronary or intravascular angiography or Doppler ultrasound has been documented in 1.5-16 % of patients (Möhlenkamp *et al*., 2002; Alegria *et al*., 2005; Kim *et al*., 2009; Ishikagawa *et al*.; 2011). It has been reported that these manifestations depend on several factors such as: the lenght and thickness of the myocardial bridge, the intrinsic tone of the artery wall, and the presence

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of loose connective tissue and adipose tissue underneath the bridge (Alegria *et al*., 2005; Saidi *et al*., 2010; Corban *et al*., 2014; Tarantini *et al*., 2016). Since patients with these anomalies are often asymptomatic, myocardial bridges were quite long considered as benign conditions. But, recently it has been observed that they can occasionally cause serious complications (Kim *et al*., 2010; Iuchi *et al*., 2013), e.g.acute coronary syndromes, cardiac arrhythmias and even sudden cardiac death (Kim *et al*., 2009; Li *et al*., 2016). The reason for the higher incidence of cardiovascular events in patients with myocardial bridges has not been elucidated yet, but some argue that the myocardial bridging may be involved in the initiation of atherosclerosis development in the segment proximal to the MB, while the intramyocardial artery segment seems to remain free from atherosclerotis lesions (Alegria *et al*., 2005; Akishima-Fukasawa *et al*., 2018; Torii *et al*., 2018). Some authors suggested that the the perivascular connective tissue might function as a protective cushion during the compression of the 'tunneled' artery segment (Saidi *et al*., 2010). Others suggest that the perivascular adipose tissue influence the blood flow through paracrine action of vasoactive substances, but might also have a proinflammatory effect and contribute to the developnment of atherosclerosis (Payne *et al*., 2012; Verhagen *et al*., 2012). There is still no real consensus regarding the best prognostic diagnostic tool and treatment (Rogers *et al*., 2017). Thus, it is important to identify appropriate parameters/criteria for clinical management of this condition.

The aim of this study was to determine the qualitative and quantitative histological features of the perivascular space connective tissue and the blood vessel adventitia, and to evaluate the relation between the thickness of this passive connective tissue compartment and the thickness of the myocardial bridge lying over it.

MATERIAL AND METHOD

A total of twenty authopsied hearts having myocardial bridges over the left anterior descending artery was collected and analysed (Table I). The existence of myocardial bridges was determined by the dissection method. MB length was measured using a precise electronic caliper (Black & Decker, 0.00-155.00 mm, Landscheid). Subsequently a tissue section was taken that included the complete 'tunnel' segment, the myocardial bridge and the associated myocardium below the tunnel segment. Transverse cuts were made along the entire length of the obtained tissue section at 0.5 centimeter-intervals.

Table I. Results of the quantitative (morphometric) analysis.

Number of samples	20
MB thickness (mean ± SD)	0,98 ± 0.44 mm
MB length (mean ± SD)	15,25±5,65 mm
PC thickness (mean ± SD)	0,58±0,22 mm

The samples were fixed in 10 % buffered formalin and embedded in paraffin. The paraffin blocks were cut into 5 micrometer-thick serial sections and stained with Hematoxylin&Eosin (HE) and Azan. Histological slides were observed using a light microscope and qualitative analysis of the blood vessel adventitia and the perivascular space structure was perfomed. Thickness of the myocardial bridge, as well as thickness of the connective tissue of the perivascular space and the blood vessel adventitia, i.e. the thickness of the passive compartment (PC) between the blood vessel media and the myocardial bridge were determined using the image processing and analysis software-ELLIPSE (Version 2, 0, 8, 1).

The thickness of the myocardial bridge was measured in the center and on both of its ends, and the mean value of these measurements was calculated. The passive compartment thickness was also measured in the center and under both ends of the myocardial bridge, and the mean value of these measurements was determined.

The study protocol was approved by the Local Ethics Committee and the Federal authorities (05-39-3910-1/18), and the study was conducted in accordance with the principles of the Declaration of Helsinki.

Statistical analysis. Values are expressed as arithmetic mean ± standard deviation. SPSS for Windows (version 13.0, SPSS Inc, Chicago, Illinois, USA) and Microsoft Excel (version 11. Microsoft Corporation, Redmond, WA, USA) were used to statistically assess the obtained data. The variables were evaluated for their normality using the Shapiro–Wilk test. For correlation analysis, the 2-tailed Pearson test was used.

RESULTS

Qualitative analysis. The connective tissue of the intermuscular compartment was of variable thickness and heterogeneous in terms of adipose and loose connective tissue abundance. It contained small blood vessels and nerves. The adipose tissue was usually located further away from the tunica media of the blood vessel, in proximity to the cardiomyocytes. It was evident that the margin between the advential tissue and the surrounding perivascular connective tissue was unclear and therefore difficult to de-

fine. In that sense, we observed the tissue of the adventitia and the perivascular connective tissue as one single (passive) compartment. This passive compartment contained mainly loose connective and adipose tissue (Figs. 1 and 2). The loose connective tissue was more compactly arranged in the area next to the blood vessel media. The connective tissue under relatively thick (large) myocardial bridges was composed predominatly of loose connective tissue with a relatively small amount of adipose tissue (Fig. 1). In contrast to that, the connective tissue in the area of relatively thin (small) myocardial bridges consisted mainly of adipose tissue with some loose connective tissue (Fig. 2). The myocardial bridges were composed of cardiomyocytes arranged in different directions (Fig. 3).

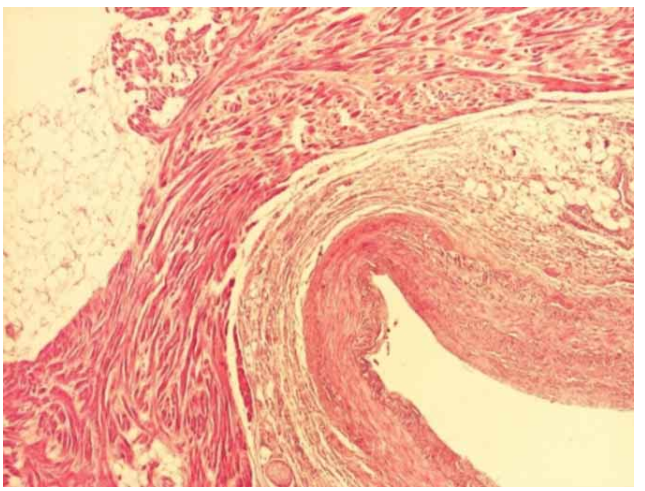

Fig. 1. Thick myocardial bridge. HE, 100X.

Fig. 2 Thin myocardial bridge; Azan; 100X.

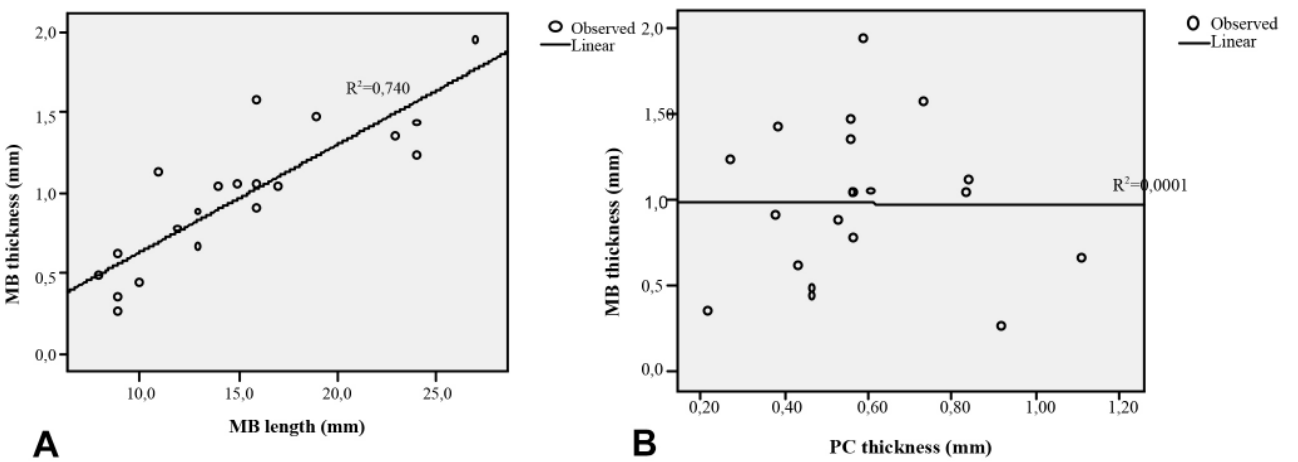

Fig. 3. A Linear correlation indicates a significant relationship between the MB thickness and the MB length (Pearson correlation, r = 0,860, p = 0,0001). B Linear correlation indicates no significant relationship between the MB thickness and the PC thickness (Pearson correlation, r = -0,011; p = 0,963) * Correlation is significant at the 0.01 level (2-tailed).

DISCUSSION

The present study aimed to determine the qualitative and quantitative features of the passive connective tissue compartment located between the blood vessel media and the myocardial bridge tissue. To our knowledge none of the previous studies was concerned with determining the quality of the connective tissue in general. Since it is quite difficult to discern the boundary between the blood vessel adventitia and the perivascular connective tissue, we observed them together as one passive compartment. Additionally, we determined the thickness of this compartment. The existence

of a myocardial bridge is considered a relatively benign condition, but in some cases it might associated with different clinical manifestations, such as myocardial infarctions or arrhythmias, and even sudden death (Kim *et al*., 2009; Li *et al*., 2016; Tarantini *et al*., 2016). These manifestations have been linked to systolic narrowing/compression of the bridged artery segment and/or atherosclerotic lesions (Kim *et al*., 2009; Ural *et al*., 2009; Li *et al*., 2016). Not all myocardial bridges cause dynamic changes in blood flow, thus, we need to distinguish two main categories: the compression-related and the non-compression-related MBs (Li *et al*., 2016). Different imaging techniques are able to detect this congenital abnormality or the disturbances in blood flow, but the detection rate differs between them (Möhlenkamp *et al*., 2002; Alegria *et al*., 2005; Tsujita *et al*., 2008; Kim *et al*., 2009; Ishikagawa *et al*.; 2011). Taking into account that myocardial bridging can be related to a deadly outcome, it is important to seek new clinical management options (Tarantini *et al*., 2016) and to identify predictive parameters to prevent serious outcomes (Rogers *et al*., 2017).

Our study has brought insights into the structure of the passive connective tissue compartment lying underneath the MB. This compartment of variable thickness (0,58±0,217 mm) was composed of variable amounts of adipose and loose connective tissue. The average thickness of the MB was 0,98 ± 0.440 mm and of the length 15,25±5,65 mm. Similar results were obtained in previous studies (Lujinovic *et al*., 2013; Gomez *et al*., 2021). We have also observed that thicker myocardial bridges were supported by relatively thin connective tissue compartments composed mainly of loose connective tissue with some adipocytes. The adipose tissue was located more superficially than the loose connective tissue, and the latter was more compact in the regions lying next to the smooth muscle cells of the blood vessel media. It was suggested that the perivascular connective tissue might function as a mattress and protect against the systolic compressive forces. The adipose tissue might also contribute in blood flow changes and atherogenesis through production of different vasoactive and proinflammatory cytokines. Some relatively recent research indicates that myocardial ischemia is not only caused by compression during systole, and filling disturbances during the diastole, but also by downstream flow limitation (Iuchi *et al*., 2013; Yu *et al*., 2021). We wondered whether the MB thickness correlated with the MB length and with PC thickness. We found a strong positive correlation between the thickness and the length of the MB ($p = 0,0001$), while there was no significant correlation between MB thickness and PC thickness ($p = 0,963$). These results indicate, and having in mind the «cushion» effect of the perivascular connective tissue underneath the MB (Saidi *et al*., 2010) and the pro-inflammatory effects of the perivascular adipose tissue (Payne *et al*., 2012; Verhagen *et al*., 2012), that in the patient evaluation, besides the MB thickness and lenght, it might be necessary to include the connective tissue compartment thickness parameter, since it might have a predictive value in context of cardiovascular events.

CONCLUSION

The passive connective tissue compartment surrounding the blood vessel media in the myocardial bridge area was mainly composed of loose connective tissue and adipose tissue in variable amounts. Thick MBs were supported by relatively thin connective tissue layers rich in loose connective tissue with some adipocytes, while the connective tissue underneath relatively thin MBs consisted predominantly of adipose tissue. The length of the MB correlates positively with its thickness, while there is no significant correlation between MB thickness and passive compartment thickness. Thus, MB and passive compartment thickness should be observed and analysed independently in the prevention of cardiovascular events and clinical management of symptomatic patients with myocardial bridging.

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RESUMEN: Los puentes miocárdicos son anomalías congénitas que se encuentran con frecuencia en las autopsias. Aunque típicamente éstos son clínicamente silenciosos, ocasionalmente se asocian con manifestaciones clínicas graves, como isquemia miocárdica o incluso muerte súbita. La fisiopatología y los factores de riesgo de estas manifestaciones aún no se han dilucidado por completo. El tejido conectivo debajo del puente se ha considerado como uno de los factores de los que dependen los síntomas. Por lo tanto, el objetivo de esta investigación fue determinar las características histológicas del tejido conectivo que se encuentra debajo del puente miocárdico y contribuir a una mejor comprensión de los efectos protectores que este compartimento pasivo podría tener en la prevención de manifestaciones clínicas graves de puente miocárdico. El estudio se llevó a cabo en veinte corazones con puentes miocárdicos. La longitud del puente se determinó utilizando un calibrador electrónico preciso. Se obtuvieron secciones de los puentes miocárdicos con el tejido conjuntivo subyacente y se prepararon para análisis cualitativo y cuantitativo. El tejido conectivo debajo de los puentes estaba compuesto de tejido adiposo y tejido conectivo laxo en diferentes proporciones. El tejido debajo de los puentes delgados estaba predominantemente compuesto de tejido adiposo, mientras que el tejido conectivo laxo era el componente dominante debajo de los puentes gruesos. Los puentes de miocardio tenían un espesor promedio de 0,98 ± 0,44 mm y

una longitud promedio de 15,25 ± 5,65 mm. Encontramos una fuerte correlación positiva entre el grosor y la longitud del puente miocárdico (r = 0,860, p = 0,0001). El grosor del compartimiento de tejido conectivo pasivo debajo de los puentes miocárdicos era de 0,58±0,22 mm, y no hubo correlación entre este parámetro y el grosor del puente miocárdico (r = -0,011; p = 0,963). En la evaluación clínica de pacientes con estas anomalías es necesario tener en consideración de forma independiente el grosor y la longitud del puente de miocardio por un lado y el grosor del tejido conectivo que se encuentra debajo del mismo por el otro.

PALABRAS CLAVE: Puente miocárdico; Tejido conjuntivo; Perivascular; Túnica adventicia.

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