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Cardiac compaction index calculated in human embryos of Carnegie stages 17-23

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ABSTRACT

Introduction: Controversies still persist regarding the events of cardiovascular morphogenesis and an almost total absence of morphometric parameters in the initial phases of its development.

Objectives: To determine the non-compacted to compacted (NC/C) myocardium ratio in both ventricles and the chronological progression of this ratio in the period studied.

Method: A descriptive, cross-sectional study was carried out with 18 human embryos belonging to the Embryoteca of the Universidad de Ciencias Médicas de Villa Clara (Cuba) classified between Carnegie stages 17 and 23. The NC/C ratio —which is simply the mathematical calculation of the ratio between the non-compacted and compacted portions per specimen and per stage— was determined.

Results: The application of this ratio in the right ventricle of the embryos obtained the following results: 7.17; 4.26; 3.12; 2.79; 2.36; 2.84 and 2.10 in Carnegie's stages 17, 18, 19, 20, 21, 22 and 23, respectively. In these same specimens, the left ventricle yielded the following results: 5.0; 3.80; 2.68; 2.18; 2.50; 2.01 and 1.56, also organized by stages.

Conclusions: NC/C ratios obtained quantitatively support a progression of the ventricular myocardial compaction in the evaluated stages; their higher values at the apex denote that it may still be incomplete in this zone.

Keywords: Human embryo, Embryonic and fetal development, Myocardial compaction, Morphometry, Compaction index

Índice de compactación cardíaca calculado en embriones humanos de los estadios 17 al 23 de Carnegie

RESUMEN

Introducción: Aún persisten controversias en los eventos de la morfogénesis cardiovascular y una ausencia, casi total, de parámetros morfométricos en las fases iniciales de su desarrollo.

Objetivos: Determinar la razón miocardio no compactado/miocardio compactado (NC/C) en ambos ventrículos y la evolución cronológica de esta razón en el período estudiado.

Método: Se realizó un estudio descriptivo, transversal, con 18 embriones huma-

Authors' contribution
MNC y MAVB: Idea and design of the research, raw data collection and analysis as well as manuscript writing and final report confection.
ECG: Idea and design of the research, final report review.
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nos pertenecientes a la Embrioteca de la Universidad de Ciencias Médicas de Villa Clara (Cuba) clasificados entre los estadios 17 y 23 de Carnegie. Se determinó el índice NC/C, el cual no es más que el cálculo matemático de la razón entre las porciones no compactada y compactada por espécimen y por estadios.
Resultados: Los resultados de la aplicación de este índice en el ventrículo derecho de los embriones son: 7,17; 4,26; 3,12; 2,79; 2,36; 2,84 y 2,10 en los estadios de Carnegie 17, 18, 19, 20, 21, 22 y 23, respectivamente. En estos mismos especímenes se obtuvo como resultado en el ventrículo izquierdo: 5,0; 3,80; 2,68; 2,18; 2,50; 2,01 y 1,56, igualmente organizado por estadios.
Conclusiones: Los índices NC/C obtenidos sustentan cuantitativamente que la compactación del miocardio ventricular avanza en los estadios evaluados; sus valores, mayores en el vértice, denotan que es posible que aún no haya concluido en esta zona.
Palabras clave: Embrión humano, Desarrollo embrionario y fetal, Compactación miocárdica, Morfometría, Índice de compactación

INTRODUCTION

The heart is the anatomical structure whose study has aroused most interest in the field of medical sciences since the dawn of humankind and, although the study of its anatomy and development has been carried out by many authors, there is still controversy regarding the events of its morphogenesis as well as an almost total absence of morphometric parameters in the initial phases of its development[1,2].

After the looping of the primitive tube takes place, at the end of the fourth week of gestation, the first trabeculations emerge, starting by the inner layers of the myocardium in the sector of its outer curvature. Morphologically, this event involves differential growth, with greater proliferation of myocytes along the outer curvature with respect to the inner curvature[3,4]. In the following weeks there is centripetal growth of the trabeculations with an increase in their surface area, which is fundamental to increase the area of gas exchange with the blood inside the cardiac tube as well as to allow a growth of the myocardial mass when there is still no specialized coronary irrigation system[5].

In the early stages of embryonic development, the cellular pool of the cardiogenic plate becomes a matter accumulation separated by lacunar and sinusoidal recesses, which give the five-week-old primordial heart tube wall a sponge-like appearance. Between the fifth and the eighth week the embryonic myocardium thickens and organizes itself, a process known as trabeculation and compaction[4,6]. Trabeculation refers to the projections of muscle tissue into the ventricular cavity, thus the inner surface stops having smooth walls and becomes trabeculated. As muscle trabeculae increase, the endocardium, originally simple, is introduced into the spaces between them covering their surface. The trabeculae with coordinated contractility and conduction stimulate the ventricles growth from the beginning, without the need for the coronary circulation to have formed[7,8].

Compaction refers to the alignment of myocytes, from a random and imprecise compression as they are in the immature myocardium, to highly compressed and coordinated myocyte bundles that function as a unit in the mature myocardium[7,8]. The compaction process allows the outer layer tissues of ventricles to proliferate and causes their growth. Grouping and compression of the basal trabeculae contributes to increase the thickness of the compacted layer, which is more pronounced in the left ventricle. Proliferation of the outer myocardial layer retains its consolidation and can extend considerably once the coronary circulation is established. The lacunar recesses (sinusoids) become intramyocardial capillaries, and the wall acquires a greater density[9].

This mechanism also seems to be responsible for the formation of the interventricular septum, which gradually increases its thickness and rises between the ventricles. After the formation of the interventricular septum is completed, remodeling of the ventricles takes place: the compacted layer is compressed and the ventricles thickness and volume increases[7,9]. It has recently been established that the interaction between the myocardium and the endocardium, which leads to the differentiation of trabeculae and myocardial layers, is controlled by the notch signaling pathway[10]. This compaction process shifts the mechanism of cell nutrition of the endo-

cardial surface to a specialized vascular system coming from the epicardial vessels and it involves in its development endothelial growth factors such as neuroregulins and angiopoietins[11].

Trabeculations are thicker in the right ventricle, which retains certain degree of trabeculation, and they are irrigated by the coronary arteries, not by the sinusoids. This layer never exceeds the compacted layer in normal hearts[12]. Failure in the course of compaction would give rise to the non-compacted left ventricle, which would explain its similarity to the embryonic heart. The evolutionary moment at which the process stops will determine the severeness of the resulting phenotype and the apex is the most affected sector because it is the last one to compact[13].

This is one of the less referenced events of cardiogenesis, a phenomenon due to which the details of the ventricular myocardium and the definitive coronary circulation are completed. It has only qualitative references since 1975[14], without a description of the process from the quantitative perspective; however, its pathological implication as a non-compacted left ventricle, which is diagnosed through echocardiography, is done through quantitative indicators. For the aforementioned reasons, the aim of this research is to determine the non-compacted/ compacted (NC/C) myocardium ratio in both ventricles as well as to determine the chronological evolution of this ratio between Carnegie stages 17 and 23.

METHOD

A cross-sectional and descriptive research was carried out, in the period from December 2016 to December 2018. Embryos belonging to the Embryoteca of the Faculty of Medicine, from the *Universidad de Ciencias Médicas* of Villa Clara were studied.

Population

Embryos in stages between fifth and eighth weeks of development were included, where it is indicated that the compaction process takes place. They were processed through histological paraffin wax technique and they were classified according to the stages system corresponding to the criteria established by the Carnegie Institute[15].

Sample

The sample consisted of 18 embryos corresponding to the seventh and eighth weeks of development, between Carnegie stages 17 and 23, in which a good quality in the histological image of the heart was guaranteed: nine of them were cross-sectionally processed, and the same number of longitudinal slices was obtained.

Technique

The sequential plane-by-plane histological study of the cardiac slices of each embryo was performed using a conventional OPTECH optical microscope (4x magnification). Of the total number of slices per embryo, those corresponding to the right and left ventricular chambers were selected, which were photographed with a Canon PowerShot G11 camera, adapted to the eye piece of the optical microscope and, from these, those that allowed a complete and better quality visualization for the morphometric study were selected.

The morphometric data of the heart were obtained using the ImageJ software, originally designed by Wayne Rasband, of the National Institutes of Health (NIH) of the United States. The program was calibrated to obtain measurements in microns (μm). For this purpose, the units were changed from pixels, which ImageJ uses, to microns, and then to millimeters (mm).

Variables

Nine variables were analyzed, three for each of the assessed heart walls:

- Lateral wall thickness of the right ventricle (compacted, trabeculated and total).
- Lateral wall thickness of the left ventricle (compacted, trabeculated and total).
- Apex wall thickness of the left ventricle (compacted, trabeculated and total)

Morphometric measurements were performed in all heart slices where the aforementioned ventricular walls were visualized (**Figure**). Seven readings for each variable to be studied were taken in each of the slices and the arithmetic mean of the measurements obtained per variable was considered. In Carnegie stages, represented by only one embryo, the maximum values of each variable expressed in mm were considered and in the stages where there was more than one specimen, the mean of all of them, in mm, was considered.

Statistical processing

The statistical package for the social sciences (SPSS for Windows version 20.0) was used and the descrip-

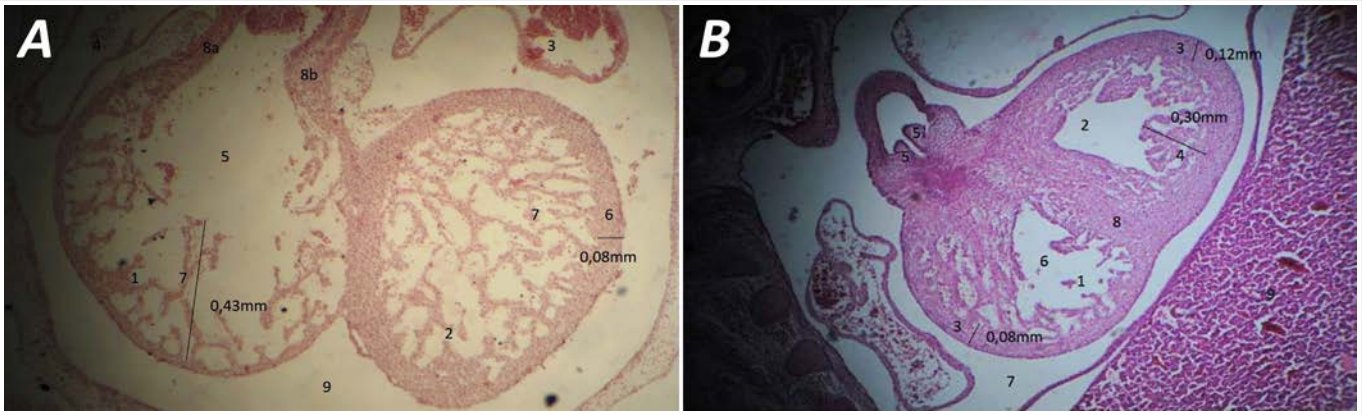

Figure. Photomicrographies of the human embryos heart slices at Carnegie stage 18. **A.** M 57-16-T Embryo: 1- right ventricle, 2- left ventricle, 3- left atrium, 4- right atrium, 5- right ventricular chamber, 6- compacted myocardium, 7- trabeculated myocardium, 8- infundibular ridges (a) right and (b) left, 9- pericardial cavity. **B.** E-24 Embryo: 1- right ventricle, 2- left ventricle, 3- compacted myocardium, 4- trabeculated myocardium, 5- outline of aortic semilunar leaflets, 6- right ventricular chamber, 7- pericardial cavity, 8- interventricular septum, 9- liver.

tive statistics per specimen was applied, where the maximum value was assumed, and these values were averaged by Carnegie stages. The NC/C index, ratio between non-compacted (trabeculated) and compacted portions, was calculated per specimen and per stage.

Ethic

The research has the permissions of the Research Ethics Committee of the Faculty of Medicine and the Biomedical Research Unit (BRU) of the *Universidad de Ciencias Médicas* of Villa Clara.

RESULTS

In an attempt to approximate the calculation of the NC/C index performed in the adult heart to detect non-compacted left ventricle, this same calculation was performed from the morphometric measurements obtained in the studied specimens. According to the adult reference, a value greater than 2.0 in echocardiographic views and greater than 2.3 in magnetic resonance imaging represents a non-compacted ventricular myocardium[10,18].

The results of the application of this NC/C index are shown in **table 1**, where it can be seen that in the right ventricle of these embryos its result was: 7.17, 4.26, 3.12, 2.79, 2.36, 2.84 and 2.10 according to Carnegie stages 17 to 23, respectively, and in the left ventricle of these specimens it was: 5.0, 3.80, 2.68, 2.18, 2.50, 2.01 and 1.56, organized by stages as well.

The table also shows the application of this compaction index to the morphometric variable in the cardiac apex with the following result: 6.0 in the stage 18, 4.80 in the stage 19, 4.43 in the stage 20, 3.52 in the stage 22 and 2.96 in the stage 23 of Carnegie.

Table 1. Application of the compaction index (not compacted/compacted) to the studied morphometric variables.

Compaction index	Carnegie stages						
	S 17	S 18	S 19	S 20	S 21	S 22	S23
Right ventricle	7.17	4.26	3.12	2.79	2.36	2.84	2.10
Left ventricle	5.0	3.80	2.68	2.18	2.50	2.01	1.56
Apex	-	6.0	4.80	4.43	-	3.52	2.96

DISCUSSION

A new form of cardiomyopathy is the non-compacted left ventricle, an intriguing condition that, although previously considered rare, it is nowadays the third most frequent type of primary heart disease in the pediatric age group, with a prevalence of 9.2% in children. It is diagnosed with an increasing frequency from the fetal age to the adulthood and it is more common in males, with a variable prevalence between males (0.015-0.25%) and females (0.014-0.14%)[16].

Nowadays there are several groups of criteria to diagnose the disease. Generally speaking, they have some points in common as well as some differences regarding the referral criteria. The absence of a true gold standard (such as a reliable genetic marker) makes it cumbersome to distinguish which criteria have the highest diagnostic accuracy. Some authors have drawn attention to the poor concordance between the different groups of criteria and the tendency to overdiagnose the disease[16].

Three groups of criteria are traditionally recognized, quoted by several authors (**Table 2**), according to Mérida Álvarez *et al.*[18].

There are differences between the listed diagnostic criteria and each of them has its limitations; however, the most widely used were published by Jenni and coworkers (quoted by Mérida Álvarez *et al.*[18]) and they are based on echocardiographic measurements performed in adults. This criterion was valuable in guiding the work and, since it coincides with other authors' considerations, it was used in this study. It is also recommended to quantify the number of non-compacted ventricular segments and to assign the respective index value to each of them, which will probably have diagnostic and prognostic usefulness. For this purpose, the left ventricle is divided into nine segments; the entire apex would be one of them and the medial and basal portions are divided into four segments each: septal, anterior, lateral and inferior[19.20]. In summary, many points of view can be adopted for the diagnosis of the disease, so in this research it was considered appropriate to join the opinion of some authors who express that, to establish the diagnosis of non-compacted myocardium, all criteria must be met, with the exception of regional hypokinesia, since the combination of all of them is highly specific[18-20].

Recent studies have found that magnetic resonance has a higher power than echocardiography when defining the extension of the anatomical disturbance, so a new diagnostic criterion has been proposed, which consists of a calculation of the ventricular mass percentage corresponding to the non-compacted layer, if it is >20% it corresponds to a non-compacted left ventricle, with a sensitivity of 78% and a specificity of 72%, but its high cost makes it used as a second-line tool, reserved for patients with diagnostic doubts[5,13,19].

Other authors propose as a diagnostic criterion an end-diastolic NC/C ratio > 2.3, which reaches high

Table 2. Diagnostic criteria for non-compacted left ventricle quoted by Mérida Álvarez *et al.*[18].

Authors/Journal/Year	Criterion
Chin and coworkers *Circulation*. 1990	• X/Y index ≤ 0.5 (where X represents the thickness of the compacted myocardium and Y, the thickness of the entire myocardium from the epicardium to the endocardial apex of the trabeculae). • Progressive increase in wall thickness (Y) and progressive decrease in the X/Y ratio of the left ventricular free wall from the basal to the apical planes. • Existence of flow between the trabeculae, within the myocardial recesses or within the fine reticular meshwork.
Jenni and coworkers *Heart*. 2001	• NC/C index > 2 (where NC corresponds to the thicker non-compacted myocardium, and C to the compacted myocardium) Predominant location in medial lateral, inferior medial and apex segments.
Stöllberger & Finsterer *J Am Soc Echocardiogr*. 2004	• Confirmation of four or more trabeculae in a same image plane and that they are located apically in relation to the insertion of papillary muscles. • The trabeculae must have the same echogenicity as the myocardium and their movement must be synchronic with the ventricular wall.

values of sensitivity (86%), specificity (99%), positive (75%) and negative (99%) predictive values[5,8,9,21]. In a study performed to 96 patients to detect non-compacted left ventricle, the NC/C index was applied, where the mean value was 2.46 versus 3.69 for patients diagnosed with non-compacted left ventricle and 1.54 for normal patients[5,20].

It is relevant to establish the diagnosis of non-compacted left ventricle because it has important prognostic implications and it also requires a management directed to specific complications[19]. The apex of the left ventricle and the inferior, anterior and lateral mid segments are the most frequently involved ones. In general, there is a global decrease in left ventricular systolic function, with a mean ejection fraction between 25-35%[8,21].

According to the values reflected when calculating the NC/C index in the study of human embryos, it can be seen that as embryonic development progresses these values decrease; that is, in embryos of Carnegie stages 17, 18 and 19 it is greater than in those in stages from 20 to 23, and its value gets closer to two, in a similar way for both ventricles.

It is also observed that in Carnegie stage 23 the result of this index calculation is ≤ 2, which implies that at this stage myocardial compaction in the lateral walls of both ventricles has been concluded; nevertheless, it is important to point out that the indexes obtained in embryos might not be identical to those obtained by other authors, given that they have been calculated in living adults and through echocardiographic images. In spite of this, the similarity of the obtained result is remarkable, which could be corroborated with a similar study in more advanced weeks of development, already in the fetal period.

The application of this index to the cardiac apex shows that the value is higher in Carnegie stages 18 and 19 in relation to 22 and 23, when it gets closer to two, but still does not reach this figure in stage 23. These results suggest that probably the apex area has not completed compaction, as suggested in the reviewed literature, from a qualitative perspective, where it is reported that the apex is the last part of the myocardium to complete this process, which ends in the eighth week of development[18-21].

The series found on the application of this index were only in adults; it has not been described in human embryos, so the results of this study may constitute an element to be taken into consideration when diagnosing this disease in early stages of life. In order to quantitatively determine, through this NC/C index, whether the myocardial compaction process in the cardiac apex ends in the Carnegie stage 23 (eighth week of the development) further studies with larger samples will be required as well as including those of the fetal period.

CONCLUSIONS

The non-compacted/compacted myocardium indexes quantitatively obtained support that the compaction of the ventricular myocardium progresses in the evaluated Carnegie stages, and that the presence of higher values in the apex point to the possibility that it has not yet concluded in this area in stage 23.

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