Resident inflammatory cells in the myocardium of children: On the way to set histologic reference standards to differentiate normal myocardium from myocarditis

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Word count: 995

Myocarditis, defined clinically and pathologically as myocardial inflammation, is thought to be the most common cause of dilated cardiomyopathy in childhood, accounting for 46% of known causes of dilated cardiomyopathy[1]. The clinical presentation can vary from asymptomatic electrocardiographic abnormalities, through fulminant acute heart failure, to sudden cardiac death. Furthermore, myocardial inflammation can be a trigger for acute decompensation in individuals with genetic cardiomyopathy and can also develop into a chronic inflammatory process. However, the diagnosis of myocarditis can be problematic, as the gold standard criteria, the Dallas criteria[2], are dependent on the availability of myocardial tissue, most commonly from endomyocardial biopsy (EMB). In children, EMB are not routinely performed in many centres due to concerns around perceived risk. Furthermore, cardiac magnetic resonance imaging, which is increasingly recognised as a useful tool for establishing the diagnosis of myocarditis and monitoring treatment efficacy, cannot be routinely performed without general anaesthesia in young children and may not be routinely available in all healthcare settings. Therefore, the diagnosis of myocarditis in young children is often a clinical one. Even when EMB is performed, there are significant limitations with the use of the Dallas criteria, including a low reported accuracy. Importantly, there are limited data on what degree of inflammatory infiltrate constitutes normality in young children.

The study by Degener et al.[3], published in this issue of the *Journal*, reports the normal inflammatory cell count in the myocardium based on a histologic analysis on 62 patients under 4 years of age who underwent planned open cardiac surgery for congenital heart disease (mainly Tetralogy of Fallot). The data were collected from 2 German hospitals from 2013 to 2017. Patients included in this analysis were mainly male (66%) with a median age of 0.5 years and more than 75% of cases were less than 1 year old. Patients with infection or inflammatory disease before surgery or with known cardiomyopathy were excluded. Samples, derived from the right ventricle (96.8%), were formalin-fixed, embedded in paraffin, and histologic and immunohistochemical analyses were performed on 4 μm thick tissue sections. Specimen were stained with hematoxylin and eosin for the detection of inflammatory infiltrates, and immunohistochemical detection with anti-CD3 antibodies were used to

identify T cells, anti-CD20 antibodies to identify B cells, anti-CD68 antibodies to identify macrophages, and anti-HLA-DR antibodies to identify activated cells. First, the Authors documented that patients included in this study had no myocarditis based on Dallas criteria (presence of inflammatory infiltrates)[2]. Second, they defined the immune cell count calculated as the number of cells per mm^2 (mean cell number counted in 5 randomly selected fields at the magnification of 200x). HLA-DR expression was graded from no expression to severe expression (0 to 3 scale).

The main result of the study was that in the heart of these young patients the median number of total inflammatory cells was 7/mm^2, of which a median of 2.5/mm^2 were $CD3^+$T cells (interquartile range was from 1 to 4), 0.5/mm^2 were $CD20^+$B cells (interquartile range was from 0 to 0.6) and 4/mm^2 were $CD68^+$macrophages (interquartile range from 2.5 to 6). Most of cells in these hearts had no expression of HLA-DR (71%), while 29% had a minor focal expression (grade 1). These data suggest that in up to 75% of infants (children below the age of 1 year) without an acute myocarditis or an inflammatory cardiomyopathy, there will be less than 11 inflammatory cells/mm^2 on an EMB. This number appears slightly lower compared to that reported in the immunohistochemical criteria by the European Society of Cardiology Working group on myocardial and pericardial disease of 14 leukocytes/mm^2 with at least 7 or more $CD3^+$T cells and 4 $CD68^+$macrophages/mm^2[4]. Specifically, the number of resident T cells in the myocardium of toddlers (children below 4 years) appears lower compared with adults based on previous expert committees[5].

Is this additional piece of information relevant in the clinical practice? Most of acute myocarditis in children are lymphocytic, while giant cell myocarditis, the histology with the poorest prognosis, is almost absent in the first two decades of life[6]. Thus, a specific histologic characterization may not be so strictly necessary in children. Nevertheless, a recent German registry of 195 children presenting with HF and myocarditis showed that up to 14% of cases needed mechanical circulatory support (MCS) and the median age of those who received MCS was 1.5 years[7]. Therefore, in this population of children below 2 years of age, the correct diagnosis is highly important, specifically to differentiate idiopathic cardiomyopathies from acute myocarditis. In

addition, recent data have suggested that desmosomal arrhythmogenic cardiomyopathies in children may present clinically as an acute myocarditis[8], highlighting the importance of an accurate diagnosis. In infants and young children, MCS requires a sternotomy, providing an opportunity for surgical biopsy of the free wall of the right ventricle. In most cases inflammatory infiltrates are evident, but, due to the patchy distribution of myocarditis, infiltrates may not be present in an individual sample. Thus, the immunohistochemical cut-offs based on the count of $CD3^+/CD68^+$cells/mm^2 derived by the Authors can provide supportive criteria for the diagnosis of acute myocarditis. A correct diagnosis can steer the treatment, specifically patients with acute myocarditis could benefit from immunomodulating therapy such as corticosteroids and/or immunoglobulins[9]. It must be noted that acute myocarditis in children can be triggered by systemic viral syndromes, in particular parvovirus B19 (PVB19), and enterovirus[10], that may also be present in the myocardium. A potential limitation of the current study is the lack of information about the viral presence in infants/toddlers without myocardial inflammation. PVB19 genome search in this population without myocardial inflammation could shed light on the topic, as it would help define if PVB19 is a bystander, or a trigger of disproportional immune response against the heart, or an active player in pediatric myocarditis.

The Authors should be congratulated on their work, which should be a source of inspiration for similar methodological studies in adults to define cut-offs for setting histologic reference standards to differentiate normal myocardium from myocarditis of inflammatory cardiomyopathies.

DECLARATION OF COMPETING INTEREST

None.

FUNDING

None.

REFERENCES

1. Towbin JA, Lowe AM, Colan SD, Sleeper LA, Orav EJ, Clunie S, et al. Incidence, causes, and outcomes of dilated cardiomyopathy in children. JAMA. 2006;296(15):1867-76. doi: 10.1001/jama.296.15.1867. PubMed PMID: 17047217.

2. Aretz HT, Billingham ME, Edwards WD, Factor SM, Fallon JT, Fenoglio JJ, Jr., et al. Myocarditis. A histopathologic definition and classification. Am J Cardiovasc Pathol. 1987;1(1):3-14. PubMed PMID: 33.

3. Degener F, Salameh A, Manuylova T, Pickardt T, Kostelka M, Daehnert I, et al. First paediatric cohort for the evaluation of inflammation in endomyocardial biopsies derived from congenital heart surgery. Int J Cardiol. 2019. Epub 2019/10/16. doi: 10.1016/j.ijcard.2019.10.006. PubMed PMID: 31611088.

4. Caforio AL, Pankuweit S, Arbustini E, Basso C, Gimeno-Blanes J, Felix SB, et al. Current state of knowledge on aetiology, diagnosis, management, and therapy of myocarditis: a position statement of the European Society of Cardiology Working Group on Myocardial and Pericardial Diseases. Eur Heart J. 2013;34(33):2636-48, 48a-48d. doi: 10.1093/eurheartj/eht210. PubMed PMID: 23824828.

5. Maisch B, Portig I, Ristic A, Hufnagel G, Pankuweit S. Definition of inflammatory cardiomyopathy (myocarditis): on the way to consensus. A status report. Herz. 2000;25(3):200-9. Epub 2000/07/25. doi: 10.1007/s000590050007. PubMed PMID: 10904839.

6. Ammirati E, Veronese G, Brambatti M, Merlo M, Cipriani M, Potena L, et al. Fulminant Versus Acute Nonfulminant Myocarditis in Patients With Left Ventricular Systolic Dysfunction. J Am Coll Cardiol. 2019;74(3):299-311. Epub 2019/07/20. doi: 10.1016/j.jacc.2019.04.063. PubMed PMID: 31319912.

7. Schubert S, Opgen-Rhein B, Boehne M, Weigelt A, Wagner R, Muller G, et al. Severe heart failure and the need for mechanical circulatory support and heart transplantation in pediatric patients

with myocarditis: Results from the prospective multicenter registry "MYKKE". Pediatr Transplant. 2019;23(7):e13548. Epub 2019/07/13. doi: 10.1111/petr.13548. PubMed PMID: 31297930.

8. Martins D, Ovaert C, Khraiche D, Boddaert N, Bonnet D, Raimondi F. Myocardial inflammation detected by cardiac MRI in Arrhythmogenic right ventricular cardiomyopathy: A paediatric case series. Int J Cardiol. 2018;271:81-6. Epub 2018/06/11. doi: 10.1016/j.ijcard.2018.05.116. PubMed PMID: 29885824.

9. Robinson J, Hartling L, Vandermeer B, Klassen TP. Intravenous immunoglobulin for presumed viral myocarditis in children and adults. Cochrane Database Syst Rev. 2015;5:CD004370. doi: 10.1002/14651858.CD004370.pub3. PubMed PMID: 25992494.

10. Matsuura H, Ichida F, Saji T, Ogawa S, Waki K, Kaneko M, et al. Clinical Features of Acute and Fulminant Myocarditis in Children- 2nd Nationwide Survey by Japanese Society of Pediatric Cardiology and Cardiac Surgery. Circ J. 2016;80(11):2362-8. Epub 2016/10/28. doi: 10.1253/circj.CJ-16-0234. PubMed PMID: 27725476.