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Diagnosing Myocarditis in Endomyocardial Biopsies: Survey of Current Practice

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Abstract

Background: Dallas criteria (DC) and European Society of Cardiology criteria (ESCC) have provided valuable frameworks for the histologic diagnosis and classification of myocarditis in endomyocardial biopsy (EMB) specimens. However, the adaptation and usage of these criteria is variable and depends on local practice settings and regions/countries. Moreover, several ancillary tests that are not included in the current criteria, such as immunohistochemistry (IHC) or viral polymerase chain reaction (PCR), have proven useful for the diagnosis of myocarditis.

Method: As a joint effort from the Association for European Cardiovascular Pathology (AECVP) and the Society for Cardiovascular Pathology (SCVP), we conducted an online survey to understand the current practice of diagnosing myocarditis.

Result: A total of 100 pathologists from 23 countries responded to the survey with the majority practicing in North America (45%) and Europe (45%). Most of the pathologists reported to examine less than 200 native heart biopsies per year (85%), and to routinely receive 3-5 fragments of tissue per case (90%). The number of hematoxylin-eosin-stained levels for each case varies from 1 to more than 9 levels, with 20% of pathologists routinely asking for more than 9 levels per case. Among the 100 pathologists, 52 reported to use the DC alone, 12 the ESCC alone, 28 both DC and ESCC and 8 reported to use neither the DC nor the ESCC. Overall, 80 pathologists reported to use the DC and 40 the ESCC. Use of DC alone is more common among North American pathologists compared to European ones (80% versus 32.6%) while use of ESCC alone is more common in Europe (20.9% versus 2.5%). IHC is utilized in either every case or selected cases by 79% of participants, and viral PCR is performed by 35% of participants. Variable terminologies are used in reporting, including both histological and clinical terms. The diagnosis of myocarditis is rendered even in the absence of myocyte injury (e.g., in cases of borderline or inactive/chronic myocarditis) by 46% respondents. The majority of the participants think it is time to update the current criteria (83%).

Conclusions: The survey data demonstrated that pathologists who render a myocarditis diagnosis practice with variable tissue preparation methods, use of ancillary studies, guideline usage, and reporting. This result highlights the clinically unmet need to update and standardize the current diagnostic criteria for myocarditis on EMB. Additional studies are warranted to establish standard of practice.

Keywords

Myocarditis

Endomyocardial biopsy

Survey

Diagnostic criteria

1. Introduction

Endomyocardial biopsy (EMB) is still considered the gold standard for the diagnosis of myocarditis, providing also important information on the inflammatory pattern. Although used infrequently, EMB could identify the etiology of the inflammatory process which is strictly linked to prognosis and to treatment [1]. EMB is clinically recommended in life-threatening clinical presentations and the reported complication rates related to the procedure are low (about 1%) [2-4]. In order to achieve optimal diagnostic accuracy and reduce sampling error, there are minimum requirements for EMBs: at least three fragments of endomyocardium need to be sampled and processed for histology; extra fragments can be used for molecular tests or ultrastructural studies, if indicated [5]. Biopsy sampling can be guided by imaging techniques in some circumstances [6,7]. Two sets of diagnostic criteria are commonly used by pathologists performing the diagnosis of myocarditis on EMB: the Dallas criteria (DC) and the European Society of Cardiology criteria (ESCC) [1,8]. The DC were introduced in 1987 with the qualitative assessment of three parameters (edema, inflammation and necrosis) and the recognition of borderline and ongoing myocarditis. Based on the Marburg consensus [9], quantitative criteria for inflammatory infiltrates coupled with immunohistochemical analysis have been adopted as standard practice in many but not all institutions since the introduction of the ESCC in 2013 [1]. In addition to histological diagnosis, ancillary testing for viral genomes in myocardial tissue through polymerase chain reaction (PCR) has been used in certain scenarios and can provide useful information for the etiological diagnosis of myocarditis. Despite these advances, anecdotal reports suggest that considerable variability may persist among pathologists in the application of the diagnostic criteria, leading to differences in the rates of myocarditis diagnosed across institutions. In recent years, reports of myocarditis occurring as a presumed consequence of immune checkpoint inhibitor therapy or coronavirus (SARS-CoV-2) infection in patients with COVID-19 illustrate the potential problems associated with poorly reproducible or inconsistently applied diagnostic criteria and further highlight the need for improvements in the histopathologic diagnosis of myocarditis. An evaluation of the current application of the DC or ESCC for myocarditis diagnosis on EMBs by cardiovascular pathologists has never been performed. This investigation was performed to assess current usage of diagnostic criteria and variations in practice among cardiovascular pathologists who routinely evaluate EMBs obtained from patients with clinical suspicion for myocarditis.

2. Methods

2.1 Scope of the survey

Between September 1 and September 16, 2021, we performed a survey of practicing cardiovascular pathologists worldwide who routinely evaluate EMBs from patients with clinically suspected myocarditis. The survey was undertaken to assess the variability in diagnosing myocarditis.

2.2 Survey creation and administration

Initial survey questions were generated by four experienced cardiovascular pathologists at academic institutions (two from European institutions and two working in the United States). The 39-question survey was sent to six additional experienced cardiovascular pathologists at academic institutions who were asked to provide feedback and suggestions. After their feedback was incorporated, the survey was pilot-tested by both the four creators and the six reviewers. No problems were reported by the pilot testers, so the survey was sent to the remainder of the study population. The targeted audience included every pathologist self-certifying as an examiner of EMBs. All surveys were administered electronically using Google Forms and the reply automatically registered at the end. Invitations to participate in the survey were delivered by the secretaries of the two main societies for cardiovascular pathology (Association for European Cardiovascular Pathology, AECVP and Society for Cardiovascular Pathology, SCVP). Additional pathologists outside these societies were asked to participate through "tweets" (delivered to pathology-related groups via the internet social media site Twitter.com) and directed emails. Participation was voluntary without compensation.

2.3 Survey content

Participants were asked to reply to a set of questions related to three main topics: general information, laboratory information and pathological diagnosis of myocarditis on EMB. The detailed list of questions is available in the supplemental file.

2.4 Statistical methods

Data are expressed as number of replies and percentage (categorical variables). To investigate the association between the categorical variables, Fisher's exact test was used. $P<0.01$ was considered statistically significant. Data were analyzed with *Jamovi project Version 2.3*.

3. Results

3.1 Characteristics of survey respondents

In total, survey results were received from 104 pathologists. Four entries were excluded because the respondents indicated they do not sign out EMBs. The 100 responses included in this study came from 79 centers (1-5 responses from each center) from 23 countries. Most of the pathologists practiced in North America (45%) and Europe (45%) (Fig. 1). Among these 79 centers, the majority represent academic hospitals (69, 87.3%), and the remainder represent non-academic hospital/private practice settings (10, 12.7%). Respondents represented a diverse range of experience and work time devoted to cardiovascular pathology. About half of the pathologists had practiced more than 20 years (51%), 16 with 5 years or less of experience, and 33 between 6-20 years. Twenty pathologists (20%) devoted more than 75% of their practice time in cardiovascular pathology, 31 between 25% and 75% (31%) and 49 pathologists (49%) indicated less than 25%.

3.2 Laboratory practice

The EMB volume varied considerably among different centers. The majority of respondents (85%) reported less than 200 native (non-transplant) EMBs processed by their institution per year (Fig. 2). A large number of respondents (88%) reported to receive formalin-fixed myocardial tissue, whereas the other 12% were given only fresh tissue. Ninety percent of pathologists routinely received 3 to 5 fragments of tissue per native EMB, and 9% typically received more than 5 fragments per case. The number of initial hematoxylin-eosin-stained levels for each case varied from 1 to 50 levels, with 20% of pathologists routinely getting more than 9 levels per case and 10% getting less than 3 levels. If the initial levels were negative for myocarditis, 51% of the pathologists request additional levels in at least a portion of the "negative" cases. A minority of the pathologists (4%) stated that the original levels were more than 20, therefore no more additional levels are ordered if the initial set is negative.

The majority (70%) of the pathologists reported performing additional histochemical stains, mainly Masson trichrome or similar for collagen staining. Thirty-five respondents applied the use of immunohistochemistry (IHC) in every case and 44 in selected cases (Fig. 3). The most common IHC stains routinely performed are CD3 (73% of respondents), CD68 (69%), and CD20 (53%) (Fig. 4). In addition, 35% of the pathologists

reported to perform viral PCR, with 27% of them applying it even in cases of negative histological findings (Fig. 5). The comparison between the two continents representing the majority of respondents (Europe and North America) is detailed in Tab. 1.

3.3 Myocarditis diagnosis approach

Among the 100 pathologists, 52 reported to use the DC alone, 12 the ESCC alone, 28 both DC and ESCC and 8 reported to use neither the DC nor the ESCC (Fig. 6). Overall, 80 pathologists reported to use the DC and 40 the ESCC. The analysis of the two most represented continents (Europe and North America) revealed that DC alone are used by 14 Europeans and by 34 North American pathologists (31.1% vs 75.5%, $p<0.01$), ESCC alone are applied by 7 European pathologists and by 1 North American (15.5% vs 2.5%, $p=0.03$) and both criteria are utilized by 20 Europeans and by 7 North Americans (44.4% vs 13.3%, $p<0.01$) (Tab. 1 and Fig. 7). Among the 40 pathologists using ESCC, 17 (42.5%) count inflammatory cells by high-power field (HPF) and 23 (57.5%) count by mm^2. For what concerns the diagnosis of "borderline myocarditis" described in the DC, 20 DC users reported that they do not apply this term in their clinical practice (20/80, 25%). Among the 60 DC users who employ the term "borderline myocarditis," 29 (48.3%) required only one cluster of inflammatory cells to render a diagnosis of borderline myocarditis. Forty-six pathologists (46%) stated that they render the diagnosis of myocarditis even in the absence of myocyte injury (e.g., in cases of borderline or inactive/chronic myocarditis).

The terms lymphocytic myocarditis, granulomatous myocarditis, giant cell myocarditis, and eosinophilic myocarditis are the most commonly used by the respondents (Fig. 8). Less than 30% of respondents reported using clinical terms such as fulminant, chronic, inflammatory, viral, autoimmune, drug-induced, bacterial, protozoal, or toxic myocarditis. Specific etiological terminologies such as COVID-19 myocarditis and immune-checkpoint myocarditis are both used by 16% of respondents. There is no significant prevalence difference of these terms by regions. One respondent (1%) mentioned to use the terminology "active myocarditis" as a diagnostic term. A total of 90 respondents (90%) routinely incorporated clinical information when diagnosing cases for myocarditis, suggesting the recognition of the clinical history on the diagnosis.

The free text response in the survey also shed light on the current practices. Some of the comments related to pre-analytical issues ("Size requirements for a single piece of tissue and number of levels to review"; "How to apply EMB criteria to LVAD cores"; "How to apply EMB criteria to autopsy specimens"; "How to approach extremely small specimens in pediatric biopsies"; "provide clinical information on pathology requisition form") and others regarded microscopic examination ("Evaluation and interpretation of edema", "provide myocyte injury grading", "Inflammatory cell quantification", "Incorporation of Brazilian Cardiovascular Society criteria [i.e. use of HLA-DR expression score in combination with lymphocyte and macrophage counts"]).

The majority of the participants (83%) think it is time to update the current criteria, regardless of their country of practice (88.4% of respondents in Europe, 73.9% in North America, 100% of respondents in Asia, and South America). Moreover, 87% of the pathologists are willing to participate in upcoming studies to update criteria for the diagnosis of myocarditis.

4. Discussion

In this study, we surveyed 100 cardiovascular pathologists from around the world about their practice of diagnosing myocarditis. We demonstrated that there is a wide range of practice, from specimen preparation, ancillary studies performed, guidelines used, and terminology/nomenclature. The results of the survey highlight the need to update the current guidelines and to unify the practice of diagnosing myocarditis on EMB specimens.

Some of the differences in practice are regional. For instance, ESCC are widely adopted in Europe but less commonly used in North America. This adaptation to different guidelines also impacts ancillary studies utilized by pathologists, as ESCC include IHC and viral PCR studies as part of the diagnostic workup, unlike the DC. Since the publication in 1987 and in 2013 of the DC and the ESCC, respectively, for the diagnosis of myocarditis in EMBs [1,8], the reporting of myocarditis remains non-standardized. No worldwide consensus was ever reached for this topic, and, as a result, wide variability in myocarditis diagnosis on EMBs exists. In this survey, many variances in laboratory procedures and in the application of diagnostic criteria appear to be related to the continent of practice. In fact, pathologists practicing in Europe reported a more frequent use of

additional levels, histochemical stains, IHC and PCR. Moreover, as expected, DC are more commonly applied by North American pathologists as compared to Europeans who replied to use more often ESCC coupled with DC.

The longstanding debate on DC has been in place since before the introduction of ESCC [10]. The publication of the ESCC based on the Marburg consensus setting the quantitative threshold of >14 mononuclear leukocytes/mm^2 on EMB samples with the presence of >7 T lymphocytes per mm^2 was meant to increase sensitivity of EMBs in myocarditis diagnosis [9]. Our survey demonstrated an uneven application of this quantitative assessment with many pathologists still relying exclusively on a qualitative evaluation as described by the DC. This diagnostic routine appears to be strictly related to the pathologists' continent of practice. Although the ESC position statement embracing the Marburg criteria acknowledges the definition of myocarditis as reported by the DC, some pathologists apply a single set of criteria (either DC or ESCC alone) and the use itself of the lymphocytes' threshold resulted in high variability according to the replies obtained in our survey.

Diagnostic challenges are related not only to the quantitative definition of inflammation, but also to the qualitative identification of cardiomyocyte injury, a histologic finding that was inconsistently used among pathologists. Several pathologists indicated that there should not be a universal requirement for myocyte injury as some of the disease entities such as hypersensitive myocarditis/eosinophilic myocarditis do not require cardiomyocyte damage to make the diagnosis [11].

Regarding the diagnostic terms reported in final reports, the inclusion of both strictly pathological and more clinically-related etiologies undoubtedly reflects the wide variability among different centers. The emergence of novel entities, such as immune checkpoint inhibitors, COVID19 and vaccine associated myocarditis that did not exist when the DC and ESCC were first established, increased attention to the grading of myocardial inflammation both in biopsy and autopsy specimens [12-15]. Careful studies of the correlation between the extent of inflammatory infiltrates and their prognosis coupled with therapeutic implications should be performed in order to create a validated grading system to avoid over- or under-diagnosing myocarditis [16-19].

The difference in the application of IHC could be related to the introduction of the inflammatory cell threshold at IHC in the ESCC, with a 55.6% vs 13.3% use of IHC in every case of suspected myocarditis in Europe vs

North-America, respectively. Clearly, the variable implementation of supplementary data obtained by IHC can significantly change the final diagnostic report.

Regarding the search for viral genomes in EMB tissue as a component of the diagnosis of myocarditis, the PCR technique was not fully developed when the DC were published in 1987. Routine testing for viral genomes in EMB specimens is performed only by one third of pathologists, with again a significant difference between Europe (53.3%) and North-America (22.3%). Despite the well-known uncertainties in interpretation of PCR results and the impact of late timing of EMB after disease onset on the sensitivity of PCR for viral detection, this technique is commonly used in centers with experience in viral genome analysis and immunosuppressive therapy [3,5].

One striking consensus of this survey, in contrast to the wide range of practice patterns, is the substantial agreement among respondents over the question of the need to update the histologic criteria for myocarditis diagnosis on EMB with 83% of affirmative answers.

5. Limitations

This survey-based analysis has all of the intrinsic limitations of the survey method including possible incomplete access to the population of concern, participant selection bias due to our modes of reaching out to the community, recall bias or participants, missing information, and collection of data at a single point in time without any possibility to measure changes in the population. The 15-day open period of the survey administration could implicate a limited sampling, but the total number of replies was deemed sufficient. Finally, the free text type options from some answers added challenges in categorizing the results.

6. Conclusion

In conclusion, in a survey of 100 pathologists, a diverse number of practices regarding the diagnosis of myocarditis were given. These inconsistencies suggest the DC and ESCC are not sufficient for practicing pathologists and that it is time to update guidelines that will be reproducible and used more consistently to render the diagnosis of myocarditis. Our survey is a preliminary action of the main societies of cardiovascular pathology, i.e. AECVP and SCVP, to start from the routine work up and reporting of EMB in different centers.

The next steps are ongoing, with ad hoc committees for both EMB and autopsy work up and reporting of myocarditis. Pathologists should provide standardized semiquantitative criteria on EMB samples, in order to be easily and globally understandable to the clinicians for the benefit of patients' care. This will improve clinical trials, make cross-institutional studies more consistent, and should ultimately help the care of patients with all forms of myocarditis.

Conflicts of Interest: none declared

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352 **Table**

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354 **Table 1**. Comparison between the replies of European and North American pathologists.

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	EU (N=45)	NA (N=45)	P
Additional levels if the initial ones are negative	30/45 (66.7)	14/45 (31.1)	**P<0.01**
Use of histochemical stains	40/45 (88.8)	24/45 (53.3)	**P<0.01**
Use of IHC in every case	25/45 (55.5)	6/45 (13.3)	**P<0.01**
Use of IHC in selected cases	20/45 (44.4)	18/45 (40)	P=0.83
Use of CD3 IHC	41/45 (91.1)	23/45 (51.1)	**P<0.01**
Use of CD68 IHC	40/45 (88.8)	19/45 (42.2)	**P<0.01**
Use of CD20 IHC	32/45 (71.1)	16/45 (35.5)	**P<0.01**
Use of PCR as ancillary test	24/45 (53.3)	10/45 (22.2)	**P<0.01**
DC alone	14/45 (31.1)	34/45 (75.5)	**P<0.01**
ESCC alone	7/45 (15.5)	1/45 (2.2)	P=0.03
Both DC-ESCC	20/45 (44.4)	6/45 (13.3)	**P<0.01**
Diagnosis of myocarditis even in the absence of myocyte injury	19/45 (42.2)	22/45 (48.9)	P=0.67

Abbreviations: PCR, polymerase chain rection; IHC, immunohistochemistry; DC, Dallas Criteria; ESCC, European Society of Cardiology Criteria; EMB, endomyocardial biopsy.

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Figure legends

Figure 1. Percent of respondents' continents of practice.

Figure 2. Number of native (non-transplant) EMB per year examined by each center.

Figure 3. IHC practice in EMB for suspected myocarditis (total and in different continents).

Figure 4. Respondents' percentage of IHC use in native (non-transplant) EMB for myocarditis diagnosis.

Figure 5. PCR testing practice in EMB for suspected myocarditis (total and in different continents).

Figure 6. Venn diagram of respondents' criteria use in native (non-transplant) EMB for myocarditis diagnosis.

Figure 7. Respondents' percentage of criteria use in different continents.

Figure 8. Percentage of reported diagnostic terms used by respondents in their practice.