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Clinical Relevance of Vasculitis in Mild form of Rejections in Heart Transplant patients

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Abstract

Background: Vasculitis had been considered a histopathological marker in severe form of both cellular and humoral rejection. However, in mild forms of rejections, it has not been considered a diagnostic and prognostic histopathologic criterion. Aim of this study is to assess clinical relevance of vasculitis, in terms of mortality and cardiac allograft vasculopathy development, in the mild forms of rejection in heart transplanted patients.

Method: We reviewed 2794 monitoring endomyocardial biopsies from 170 adult heart transplanted pts at our center. On endomyocardial biopsies, we evaluated the presence of vasculitis in the different types of rejection. Clinical data were collected during follow up. A Multi-State Markov model was applied to describe the rejection dynamic profile and hazard for mortality, persistence of rejection and CAV development.

Results: Vasculitis was detected in 442 endomyocardial biopsies (442/2794, 15,8%) with an incremental percent distribution according to the severity of rejection. In ACR, vasculitis significantly increased the risk of mortality by 9%, if the patient was negative for ACR, and by 14% in a mild form of rejection. Similar results were obtained for pAMR and mixed rejection. In ACR, pAMR and mixed rejection patients with vasculitis have a shorter life expectancy than patients without with a loss of four months at ten year follow up for ACR, and of 9 months for pAMR and mixed rejection.

Conclusions Vasculitis when present, independently from the grade and type of rejection, carries a negative prognostic value. The worse histopathological feature was represented by the association of pAMR+ACR and vasculitis.

Keywords: vasculitis, arteriolitis, acute cellular rejection, mixed rejection, antibody mediated rejection, heart transplantation, EMBs, predictive modelling

Introduction

In the current era, despite considerable improvements in the diagnosis of rejection and immunosuppression therapy, chronic cardiac allograft rejection remains the major factor leading to graft failure. Endomyocardial biopsy (EMB) remains the gold standard for the diagnosis of rejection in Heart Transplanted (HTx) patients. Even though acute cellular rejection (ACR) is better controlled by the immunosuppressive therapy,the humoral or antibody-mediated rejection (AMR) remains challenging for diagnosis and treatment.

The concept of vascular damage has evolved during the years, starting in 1990 with the first International Society for Heart and lung Transplantation (ISHLT) pathological classification criteria for the diagnosis of acute cellular rejection, in which the vascular damage was described as endothelial damage associated with edema and vasculitis in the more severe cases and associated with humoral rejection1-3 (Billingham E et al 1990). . In 2004, ISHLT revised the pathological classification and the histological features of humoral rejection were listed among the pathological criteria4 (Stewart 2004). Only in 2013, the new ISHLT pathological classification of humoral rejection introduced a new concept of vascular damage as an on-going phenomenon and recognized and classified different pathological grades, in the attempt to improve the diagnosis of AMR5 (Berry GJ 2013).

In the quest of personalized medicine, the introduction of molecular markers6,7 (Duong van huyden 2014, Di Francesco 2018) and gene profiling 8-10(Hidalgo 2008, Loupy A 2017, Halloran PF 2017) have been considered as a companion tool for better defining the complexity of AMR and helping clinicians in the treatment of this condition.

Despite considerable improvements, the pathogenesis and pathophysiology of AMR remain unclear and many histological features are not completely clarified. Although vasculitis of the intramyocardial arteriolae (defined as the presence of smooth muscle cells in the parietal wall with a diameter ranging between 150-200 micron) has been described in more severe cases of rejection both in ACR and AMR, it is also often observed in mild ACR and associated with capillaritis in pAMR on the same EMBs. The present study aims to assess the clinical and prognostic relevance of vasculitis, in terms of mortality and Cardiac Allograft Vasculopathy (CAV) development and focusing in particular on the mild forms of rejection in HTx adult patients.

Materials and Methods

In our study we considered all adult patients that underwent heart transplantation at the cardiac center "Gallucci" of the Hospital-University of Padua in the period from January 2008 to December 2017 considering the follow-up until December 2017.

Monitoring EMBs were performed according to the surveillance protocols11 (inseriamo una nostra ref che cita il protocollo) adopted in our center. For each individual patient, we collected the demographic data, the cause of transplant, the donor data,, and eventually the cause of death, and for each biopsy the immunosuppressive therapy, the pathologic diagnosis, and the presence of circulating DSA.

The DSA assay was performed in the suspicion of AMR, mixed rejection and in the follow up of patients with already positive antibody detection.

All biopsies have been reviewed at light microscopy to evaluate the presence or absence of vasculitis. In general, vasculitis was defined as an inflammation of arteries, capillaries, and veins,

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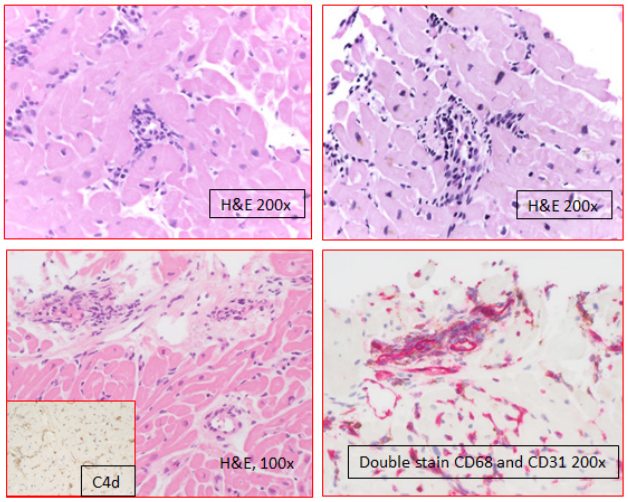
In particular, arterial vasculitis as the presence of inflammatory cell infiltration in the arterial wall of intramyocardial arterial vessels present on EMBs. Intramyocardial arterial vessels range in diameter from 150-200 microns to 20 microns when the capillaries start and are characterized by the presence of smooth muscle cells in their parietal wall.



ACR and AMR were reclassified according to the classifications of ISHLT 1990/2004 for ACR4 and 2013 for AMR5 (….). All biopsies performed before 2013 have been reclassified according to the current criteria.

We excluded from the study all pediatric patients (less than 16 years), all patients with suspected infection and PTLD, all patients who died before any monitoring EMBs or within one month after transplantation.

The detection of DSA pre and post-transplantation was based on the Luminex technology for antibody screening of HLA classes I and II.

For the CAV evaluation, coronary angiography was performed in all patients one year after transplantation.

Statistical analysis:

Multi-State Markov model

The MSM model is a statistical method that is used to describe stochastic processes where individuals move from one health condition to other health conditions12, 13 [7, 8]. Once the possible clinical statuses in which a patient can transit are defined, the model allows the estimation of several statistical summaries that provide information on how patients move from different conditions. For example, it can be of interest to know the 1-year mortality of a subject with mild forms of AMR rejection. Furthermore, it can be meaningful to evaluate how the presence of vasculitis affects the risk of CAV in patients with mild forms of ACR rejection. The main advantage of this method is that it accounts for the dynamic nature of the statuses observed at each biopsy during the follow-up and it does not consider that each status is identical and can be experienced at any time, as in standard survival analysis. Hence, it allows for the representation of multiple ordered events per individual.

Model's structure

The model was structured defining 5 statuses that represent clinical conditions that an individual can experience during the follow-up: 1) Absence of rejection; 2) Mild form of rejection; 3) Moderate-severe form of rejection; 4) CAV; 5) Death. Three models were applied, one for each type of rejection: ACR, pAMR and mixed (MR). Mild and severe form of rejection were defined as 1R and 2R or 3R, respectively, in ACR, whereas they were defined as pAMR (1H+) or (1I+) and pAMR 2 or 3 in pAMR, respectively. Mixed rejection was defined as the concomitant presence of pAMR>0 and ACR>1. Death was considered the "absorbing status", i.e. the status from which an individual cannot move from. The structures of the models are shown in Figure 1. Arrow and edges represent the transition from one status to the other. Given the high number of statuses and possible transitions compared to the number of subjects in the sample, the model was defined in such a way that only the most relevant transitions from a clinical point-of-view were evaluated.

In this study, the transitions are only known to occur in a bounded time interval but the exact date when the subject moves from one status to the other is not known. Furthermore, the number of biopsies and the time when they were performed may vary across patients. For these reasons, we used a time-inhomogeneous MSM model that fits the data collection process of the study, a method that accounts for the eventual misclassification of the observed statuses at each time point 14[9]. The presence of vasculitis was used as an explanatory variable in the model in order to understand how vasculitis changes the hazard ratio of moving from an initial status to another. The 95% Confidence Intervals (CIs) associated to each parameter were computed with the Bias-Corrected and Accelerated bootstrap (BCa) technique using 2000 replications [10]15.

Registry data analysis

The estimates obtained in the model were adapted to a reference target population of patients who underwent HTx to provide a better characterization of the role played by vasculitis on the prognosis of the subjects. We used as reference population the registry data of patients who underwent HTX from 2004 to 2018 provided by the International Society for Hearth and Lung Transplantation (ISHLT) Registry. The estimates of the MSM models were applied to the registry data to project the survival curves of an average subject of the target population assuming absence and presence of vasculitis. The estimates were adjusted to match age and sex distribution of the sample with that of target population. The impact of vasculitis on the prognosis of HTx patients was expressed with years of life lost at different time-points of the follow-up.

All the statistical analyses were performed with R statistical software (version 3.6.3) 16[11]. The MSM model was fitted using msm R package (version 1.6.7) 17[12].

Results

Study population

One hundred and seventy heart transplanted patients were evaluated, 42 women (24%) and 128 men (75,3%), with a median of 57,37±12.9 years (range 17,9-74,6 years), 50±11.2 years for women and 59±13.3 for men. (Table 1)

Patients have been followed for a median of 4.9 years (range 0, 22-10, 03 years). During this time period 2794 monitoring EMBs were collected with an average of 15 biopsies for patient.

Of the 2794 EMBs, 1599/2794 (55, 6 %) were negative for rejection (ACR0 and pAMR0), 1037/2794 (37%) showed mild ACR, 158/2794 (5,6%) severe ACR, (table 2), 146/2794 (5,2%) pAMR, and 100/2794

(3.8%) had both ACR and AMR.

In 140 of 170 patients (82, 4%) at least one episode of vasculitis has been reported at histological evaluation. Acute cellular rejection (ACR) CR was present in 54.8% (76/140) of patients with vasculitis, pAMR in 7.4% (12/140) and ACR+pAMR (mixed rejection, MR) in 37.8% (52/140). Positive pAMR(>0) was found in 38.8% of patients (66/170), of which only 3 were found to be symptomatic and were treated with immunosuppressive therapy, specific for humoral rejection. Of the 170 patients, 53% (91/170) had at least a DSA testing, and 39 resulted to be positive. In total, 241 DSA testing were performed with a positivity in half of them, (49.8%) (120/241) (Table1).

Overall, vasculitis was detected in 442 EMBs 442/2794 (15, 8%). It was present in 355/1037 (34%) with mild ACR (1R), increased to 52% (71/138) in moderate ACR (2R), and to 70% (14/20) in severe ACR (3R). (Table 2).

In the group of EMBs with pAMR >0 (146/2794, 5.5%) the vasculitis was found in 53/146 (36.1%), in particular in 13/28 (46.1%) of EMBs with pAMR 1H +, in 27/78 (34.6%) with pAMR 1(I +), in 10/36 (27.8%) with pAMR 2 and in 3/4 (75%) of EMBs with pAMR 3. (Table 3)

In the group of EMBs with Mixed rejection the distribution of vasculitis was 1001/144 (69.25%), with a percentage of 74/1001 (74%) with ACR 1R, 23/1001 (23%) with ACR 2R and 3/1010 (3%) with ACR 3R.

Twenty six/170 (15%) patients died during follow-up.CAV in 8/26 (30, 8%), multiorgan failure in 5/26 (19, 2%), infection in 5/26 (19, 2%) and other causes in 8 /26 (30, 8%).

Multi-state Markov model

Table 4 shows the effect of vasculitis on the transitions from one status to another estimated by the MSM models. Estimates are also represented in Figure 2. The presence of vasculitis had a negative impact on the prognosis of the HTx patients in all the three types of rejection.

Regarding ACR, the vasculitis significantly increases by 9% (HR [95% CI]: 1.09; [1.00-1.18]) the hazard of mortality if the patient was negative for ACR and by 14% (HR [95% CI]: 1.14; [1.02-1.37]) if the patient had a mild form of rejection (1R). Moreover, vasculitis increased the hazard of rejection by 63% even in the subclinical form of ACR. (HR [95% CI]: 1.63; [1.25-2.04]).

The presence of vasculitis has a higher negative impact on prognosis in pAMR rejection than in ACR rejection, with significant increased hazard of mortality, if the patient was negative for pAMR rejection (HR [95% CI]: 1.38; [1.07-1.72]), and if the patient had a mild form of rejection (HR [95% CI]: 1.35; [1.23-1.46]). Furthermore, patients with vasculitis have a significant higher hazard of moving from negative pAMR rejection to a mild form of pAMR rejection (HR [95% CI]: 1.85; [1.15-2.54]), and to a severe form of pAMR rejection (HR [95% CI]: 1.44; [1.07-1.91]).

Similar results were obtained in mixed rejection: vasculitis increased by 38% (HR [95% CI]: 1.38; [1.08-1.71]) and 37% (HR [95% CI]: 1.37; [1.30-1.46]) the hazard of mortality in a patient negative for mixed rejection and presented a mild form of mixed rejection, respectively. In addition, patients with vasculitis have a significant higher risk of moving from no mixed rejection to a severe form of mixed rejection (HR [95% CI]: 1.75; [1.20-2.40]) than patients without vasculitis. For all three types of rejection, the vasculitis did not have a significant impact on the prognosis in terms of CAV.

ISHLT Registry data analysis

On average HTx patients with vasculitis had a shorter life-expectancy than HTx patients without vasculitis. Figure 3 shows the survival curves obtained by projecting the MSM's estimates for mild form of rejection to the ISHLT registry data.

Regarding mild form of ACR rejection, a HTx patient with vasculitis have a life-expectancy of 0.11 years (95% CI; 0.02-0.3) lower than a HTx subject with vasculitis at 5-years after heart transplantation, i.e. the absence of vasculitis will save on average more than 1 month of life at 5 years of follow-up. Ten-years after HTx, a patient with vasculitis will on average live 0.33 years (95% CI; 0.06-0.77) less than a patient without vasculitis, i.e. 4 months of life loss.

Life-expectancy of patients with vasculitis is further reduced in both mild form of pAMR and mixed rejection. With mild pAMR rejection, a patient with vasculitis is expected to lose 0.29 years (95% CI; 0.19-0.37) of life compared to a patient without vasculitis at 5-years follow-up. Similar results were obtained for mixed rejection (0.3 years of life lost [95% CI; 0.24-0.37]). At 10 years follow-up, a HTx patient with a mild form of pAMR rejection will live on average 0.74 years (95% CI; 0.5-0.95) less if vasculitis occurs, which means that she/he will lose almost 9 months of life. Results were similar for mild form of mixed rejection: the presence of vasculitis decreases of 0.78 years (95% CI; 0.63-0.94) life-expectancy.

Discussion

The novelty of our study was that patients with vasculitis even in the setting of mild rejection experience, worse prognosis in terms of mortality and persistence of rejection. Moreover, persistence of rejection occurred more frequently in patients with vasculitis regardless of type of rejection. Vasculitis is confined to the vascular damage targeting intramyocardial arteries up to capillaries. In the classifications of ACR vasculitis is mentioned as a morphological criterion in the severe grade 3B/4 or 3R4 (…) that are an uncommon finding in monitoring endomyocardial biopsies. Vasculitis in AMR is mentioned as histopathological criteria in pAMR3 (….). In the lower grades of ACR and AMR vasculitis or arteriolitis is not mentioned. Some authors 18, 19(Fedrigo m AMJ della Bonato, Miller D case con ptld) described the lymphoproiferative disorders as vasculitis.

In the early days of heart transplantion only few groups identified to arteriolar vasculitis on EMBs as a histological predictor of poor outcome, mainly associated to acute cellular rejction20,21 (herskowitz a, 1987, smith sh 1987). . Higuchi in 199922 suggested a possible relationship of concomitatnt intramyoardial end epicardial vasculitis in heart transplant with development of cardiac allograft vasculopathy. More recently Cipullo et al identified the presence of vasculitis on EMBs as an independent predictive factor of ACR and worse outcome in the setting of ACR23, 24 (Cipullo et al 2011 e 2018). They adopted a grading score for inflammatory infiltrate of arteriolar wall on EMBs and showed that mild to moderate vasculitis had a 4-fold higher chance to develop acute cellular rejection than pts without. Severe vasculitis had a ten-fold higher chance to develop acute cellular rejection. However, the predictive value of development of ACR was found only in severe vasculitis. In a further study they showed that gene expression profile by RT-PCR of endothelium damage was related to vasculitis with poor outcome24 (Cipullo R 2018).

In our study, we applied the MSM model since it allows considering rejection as a dynamic process that characterizes the history of patients and their outcomes. The choice of the statistical method was motivated by its ability to describe all the possible histopathological features that a patient can have after HTx. Basically, it considers each grade of rejection (ACR and pAMR) with or without vasculitis at the time of the biopsy as "status" of the patient. By evaluating how many transitions occurred from one status to another, the statistical approach can estimate the hazard of experiencing events such as death or CAV from each defined status and how the presence of vasculitis can impact on such hazard.

Vasculitis associated with mixed rejection is the worst condition that patients can experience in comparison with all types of rejection without vasculitis. Many studies showed that mixed rejection is associated with the worst outcome 25-27(kfoury a 2015, 2016, Kobashigawa J) compared to ACR and AMR. This reflects massive inflammatory response in the graft. This suggests a strong relation between intramyocardial arteriolitis and epicardial coronary arteries that develop CAV. The concomitant histological evidence of intramyocadial and epicardial vasculitis has already been reported in a necroscopy study of HTx pts evaluating the coronary arterial tree, strengthening the relation between vascular inflammation and CAV development22. (Higuchi MI 1999). The concept of inflammatory burden as a diagnostic and prognostic marker of AMR on EMBs and the interplay between cellular components of ACR and AMR was put forward by European pathology group in 2015. The Inflammatory burden concept has modified the previous belief that AMR was spared by inflammatory cells infiltration. It is now clear that microvascular inflammation is associated with worse prognosis28 (Fedrigo M 2015). Even in other organs the increasing inflammation, even though confined to capillaries, is associated with worse prognosis. Inflammation causes changes in the endothelium phenotyping of all the arterial vessel tree (both intramyocardial and epicardial vessels) and produces similar epitopes triggering and amplifying the recruitment of inflammatory cells and the secondary endothelial damage.(……). In our study we could not detect a significant impact of vasculitis on CAV development. The reason for that could be searched in the time assessment of CAV development that per protocol is performed with angiography at 1 year. This time span can be too short to show the development of CAV.

In conclusion, vasculitis should not be considered a trivial histological feature but should be regarded as an important complementary marker in scoring the EMBs for diagnosis and opens new therapeutic and diagnostic perspectives. Vasculitis is an histological features easy to be detected on EMB which should be reported and considered for a better management of the patient, should alert the clinician that the inflammatory state of the pts is more severe than in its absence and should require a more strict surveillance and a better immunosuppressive management.

Study limitations

The sample size of our study limited may question the generazibility or transferability of our findings. Future studies with a higher number of enrolled patients should be conducted to further investigate the impact of vasculitis on the prognosis of HTx patients.

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