



The Diagnosis of Antibody-Mediated Rejection (AMR) and Acute Cellular Rejection (ACR) in Transplanted Hearts - What We Already Know and What We Should Get to Know. The Experience of Polish Single Centre Population

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Abstract

Background: New ISHLT 2013 criteria requires further study on significance of histopathological changes in Endomyocardial Biopsy (EMB). Guidelines concerning optimal EMB quality for rejection assessment are sparse in literature. The aims of our study, were to assess the significance of histopathological changes in diagnosis and monitoring of AMR as well as to determine the influence of number and size of myocardium in diagnosis of ACR in EMBs of transplanted heart in Polish group of patients after Htx.

Material and methods: 1350 EMBs from 212 patients after Htx were diagnosed as AMR1 and AMR0, according to ISHLT 2004 criteria. In all EMBs histopathological features suggestive of AMR were described. The frequency of each change was compared between groups. The number and size of specimen were categorized as follow: 1) Above three myocardial samples > 5 mm in size; 2) Two-myocardial samples 2-5 mm² in size; 3) One myocardial samples < 2 mm² in size; 4) Undiagnostic EMB. The frequency of each ACR grade was assessed depending on myocardium quality.

Results: 121 EMBs from 20 patients with AMR1 (group 1), and 1229 EMBs from 192 patients with AMR0 (group 2), were analysed. Endothelial swelling and intracapillary activated mononuclears were found statistically more often in patients with C4d deposits, $p < 0.001$. 24 (1.74%) EMBs were undiagnostic for ACR diagnosis and thus they were not included into analysis. ACR of any grade was statistically more often diagnosed in EMBs from category 1 and 2 than category 3 ($p < 0.001$).

Conclusion: Intracapillary activated mononuclears and endothelial swelling seem to be more specific for AMR diagnosis than isolated interstitial edema. To diagnose ACR at least two samples of myocardium of minimal surface 2 mm² are needed.

Keywords

Heart transplantation, Antibody mediated rejection, Intracapillary activated mononuclears, Endothelial swelling, Interstitial edema, Cellular rejection, Endomyocardial biopsy, Sample quality

Introduction

Antibody Mediated Rejection (AMR) is a significant clinical and diagnostic problem in patients after Heart Transplantation (Htx). Its mechanism and impact on allograft survival have not been completely recognized. It is believed that AMR may have unfavorable influence on allograft function and survival, increase risk of sudden cardiac death and precipitate development of Cardiac Allograft Vasculopathy (CAV) [1-4]. International Society for Heart and Lung Transplantation (ISHLT) in first criteria for reporting heart rejection (1990) did not

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distinguish AMR as separate diagnosis [5]. It was considered as supplementary finding. Precise diagnostic criteria were created in 2004 [6] and they included: clinical symptoms such as heart dysfunction of unexplained origin, presence of Donor-Specific Antibodies (DSA) and changes in Endomyocardial Biopsy (EMBs).

Histopathologic features suggestive of AMR consist of endothelial swelling, interstitial edema and intracapillary activated mononuclear. Immunohistochemical markers supporting AMR diagnosis are C4d positive staining and presence of CD68 (+) macrophages within capillaries. Further studies proved that even clinically silent and sub-silent cases of AMR can cause earlier cardiovascular mortality [7,8] thus it seemed that humoral rejection was rather a process. Based on those findings, ISHLT proposed new AMR classification [9] in 2013. Although many problems concerning diagnosis of humoral rejection were fixed, some controversy, related primarily to histopathological changes, remained. New criteria apply only for AMR diagnosis, grading of acute cellular rejection has not been changed. Both kinds of rejection depend on EMB quality, but ACR in particular due to its focal character. Initial ISHLT 1990 criteria [5] suggested that number of myocardial samples should be at least four pieces. Revised classification [6] reduced the number to three fragments containing minimum 50% of myocardium. Optimal surface of material necessary for ACR diagnosis has never been studied. The primary aim of our study was to assess the significance of each histopathological change in diagnosis and monitoring of AMR. Secondary aim was to determine the influence of number and size of myocardium in diagnosis of ACR in EMBs of transplanted heart in Polish group of patients after Htx.

Material and Methods

1350 EMBs from 212 patients who underwent heart transplantation in years 2001-2013 and they survived period of 30 days after operation were enrolled to the study. All EMBs were retrospectively examined in the Department of Pathology, The Children's Memorial Health Institute by three qualified pathologists unaware of clinical data. They consisted mostly of protocol EMBs, including repeated ones due to ACR diagnosis (verification). Endomyocardial specimens were taken from right ventricle, fixed in 4% formalin and embedded in paraffin. Special method of quick paraffin treatment was then applied. The paraffin blocks were cut for 4 µm slices stained with Hematoxylin/Eosin (HE). Immunohistochemical (IHC) staining with polyclonal antibodies against C4d (Biomedica groupe, dilution 1:40) was performed in all diagnostic EMBs as the marker of Antibody-Mediated Rejection (AMR). Features indicative of AMR were summarized as capillary deposition of C4d of the myocardium (> 50% vessels involved). The staining of venular, ar-

terial, or arteriolar endothelial cells, arterial elastic lamina, the capillaries in Quilty effect were not considered to be indicative of AMR. CD68 stain was not performed in our Department. Histopathologic changes including endothelial swelling, interstitial edema and intracapillary activated mononuclears were assessed in each EMB and were classified according to new ISHLT 2013 classification as pAMR0, pAMR 1h, pAMR1i, pAMR2 and pAMR3. Acute Cellular Rejection (ACR) was diagnosed according to ISHLT 2004 criteria as ACR0, ACR1 and ACR2. In biopsies with presence of inflammatory infiltrate that did not meet ISHLT 2004 criteria, category 1r (minimal changes) was created because we believe that from histopathological point of view previous classification (ISHLT 1990) [10] was more accurate. The number and size of specimen were also evaluated and categorized as follow: 1) Above three myocardial samples > 5 mm in size; 2) Two- myocardial samples 2-5 mm² in size; 3) One myocardial samples < 2 mm² in size; 4) Undiagnostic to determine the influence of number and size of myocardium in diagnosis of ACR EMB. The frequency of ACR diagnosis was assessed depending on myocardial quality which means that the frequency of each rejection grade was assessed in each size category (small, medium, big) and then compared using appropriate statistical analysis (chi-squared test). The difference of p value < 0.05 was statistically significant and was recognized as cut off point for minimal size and number of EMB mandatory for reliable ACR diagnosis.

Results

121 EMBs from 20 patients with AMR1 (group 1), and 1229 EMBs from 192 patients with AMR0 (group 2), were analysed. Endothelial swelling was observed in 47 (38.84%) EMBs from group 1, in 195 (15.86%) EMBs from group 2, $p < 0.001$. Interstitial edema was present in 42 (34.71%) EMBs from group 1; in 433 (35.23%) EMBs from group 2, $p = 0.988$. Intracapillary activated mononuclears were found in 27 (22.31%) EMBs from group 1; in 83 (6.75%) EMBs from group 2, $p < 0.001$. There was no significant difference in frequency of histopathological features when EMBs were analysed according to time after transplantation. The number of EMBs in each patient varied from 1 (10/4.71% of patients) to 15 (1/0.47% of patients); the average number was 6 EMBs. 24 (1.74%) EMBs were undiagnostic and thus they were not included into analysis. 958 (70.96%) EMBs belonged to category 2; 256 (18.96%) EMBs to category 1; 136 (10.07%) EMBs to category 3. ACR grade 1R was diagnosed in 265 (18.96%) EMBs; grade 2R in 60 (4.44%) EMBs; minimal rejection that did not fulfill ISHLT criteria - grade 1r in 69 (5.11%) EMBs; in 956 (70.81%) EMBs there was no rejection; ACR grade 3R was not found. ACR of any grade was statistically more often diagnosed in EMBs from cat-

Table 1: The frequency of each histopathological change in EMBs of 212 adult patients after heart transplantation.

Category	Endothelial swelling	Activated intracapillary mononuclears	Interstitial edema	Total
Group C4d + (number of EMBs)	38.8%	22.3%	34.7%	16 (7.5%) patients 121 (9%) EMBs
Group C4d - (number of EMBs)	15.9%	6.7%	35.2%	
p-value	< 0.001	< 0.001	NS	

NS: Not Significant.

Table 2: Demographic characteristic of studied group.

Category	
Sex	40 (18.9%) women and 172 (81.1%) men Total n = 212
Age	From 17 up to 82-years-old
Period of heart transplantation	Between 2001 and 2013

category 1 and 2 than category 3 ($p < 0.001$). The difference between category 1 and 2 was minor.

The frequency of each histopathological change in EMBs of studied group is presented in [Table 1](#).

Brief demographic characteristic of the group is presented in [Table 2](#).

Discussion

New ISHLT 2013 criteria for reporting AMR in endomyocardial biopsy are more precise and specify many problems related to humoral rejection. Primary and secondary Immunohistochemical (IHC) panels were proposed, histopathological changes were regarded as fundamental for diagnostics of humoral rejection. Nonetheless, there are some controversy concerning the significance of certain microscopic findings. It was not defined whether category pAMR1h can be diagnosed when one out of three changes is found or all lesions should be present. It has not been determined so far if each change is equally specific for AMR diagnosis. The extensiveness of changes suggestive for humoral rejection were also not established. These points seem to be important, specially that recent study by Hammond, et al. proved that pAMR1h, pAMR1i, and pAMR2 have similar risks to develop cardiovascular mortality [11]. Therefore, proper selection of patients is a key diagnostic task. In our study, were evaluated 1350 EMBs from 212 adult patients who underwent heart transplantation in years 2001-2013 and they survived period of 30 days after operation. Two groups, based on C4d positivity were created and histopathological changes were assessed in each biopsy. Our results revealed that intracapillary activated mononuclears and endothelial swelling were statistically more often found in EMBs of patients with C4d positive staining. We did not obtain such a result for interstitial edema. There was no significant difference in frequency of histopathological features when EMBs were analysed

according to time after transplantation. Our results are concomitant with previous paper by Hammond, et al. [12], who proved that specificity of intracapillary activated mononuclears and endothelial swelling were satisfying for humoral rejection diagnosis. The sensitivity of the changes were 63% and 30%, respectively. According to some authors [1] presence of singular lesion is not a sufficient descriptor of pAMR1h. Based on our results and experience, it refers to isolated interstitial edema in particular. In our group we diagnosed pAMR1h if at least two out of three changes were found. It seems reasonable to describe microscopic findings when they are present in more than 50% of specimen. Clinical data, specially CMV infection, are of great importance because AMR is not the only factor causing such lesions. However, in our group, CMV infection occurred sporadically and never simultaneously with any changes in the biopsy. Quality of EMB is another diagnostic problem. Both kinds of rejection depend on specimen size, but ACR more significantly due to its focal character. Therefore, our secondary goal was to determine the influence of number and size of myocardium in diagnosis of ACR. According to our best knowledge it is first study applying to this issue. We categorized samples into four groups ([Table 3](#)) from the biggest ones to undiagnostic material. Statistical analysis proved that at least two samples of myocardium of minimal surface 2 mm² are needed for ACR of any grade diagnosis. We decided to focus not only on number of specimen but also total surface of myocardium. In everyday practice we sometimes deal with EMBs containing mostly fibryn and thrombi thus one big piece of myocardium may be more diagnostic than four samples comprising of fibrotic tissue. Initial ISHLT 1990 criteria [5] suggested that number of myocardial samples should be at least four pieces. Revised classification [6] reduced the number to three fragments containing minimum 50% of myocardium. We should remember that within years the size of catheter has changed, now a days it is smaller thus surface of obtained samples is also reduced. We observed it in our material, EMBs before 2004 were bigger than the later ones. The lifespan of patients after Htx is increasing and number of performed EMBs as well. Catheterization favours fibrosis therefore new diagnostic methods are being inquired [13]. None of them has been specific enough for diagnosis of allograft rejection and

Table 3: The frequency of each ACR grade (ISHLT 2004) according to EMBs quality.

ACR grades	EMBs (%) quality				Percentage of ACR in all group
	Big samples	Middle samples	Small samples	Not diagnostics material	
1R (minimal)	24.5% EMBs	70.9% EMBs	4.5% EMBs	0% EMBs	19% EMBs
2R (mild)	35% EMBs	58.3% EMBs	6.7% EMBs	0% EMBs	4.4% EMBs
3R (severe)	0% EMBs	0% EMBs	0% EMBs	0% EMBs	0% EMBs
Minimal ACR	29% EMBs	66.7% EMBs	4.3% EMBs	0% EMBs	5.1% EMBs

EMB remains golden diagnostic standard. It seems that finding less invasive and less expensive procedure will be important future goal. ISHLT recently focused on mixed AMR-ACR rejection. ACR has been studied thoroughly and complete recognition of humoral rejection mechanism and its proper diagnosis is of great importance now a days. The limitations of our study are facts that we could not prove AMR with DSA assessment and we did not perform CD68 staining which was mandatory in previous classification.

Conclusion

Intracapillary activated mononuclears and endothelial swelling are statistically more often find in EMBs of patients with AMR, while isolated interstitial edema seems not to be sensitive enough for AMR diagnosis and monitoring. To diagnose ACR at least two samples of myocardium of minimal surface 2 mm² are needed.

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