Peer Review File

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Reviewer A

This is a very interesting paper highlighting the use of echocardiography alone versus endomyocardial biopsy in surveillance for acute rejection post transplant. I believe the authors do a nice job in discussing the possible limitations of these cohorts and the fact that there is no way to discern AMR without biopsy. I think these are interesting results and novel to the field.

Reply : We would like to thank reviewer A for his comments.

Reviewer B

This important study aimed to evaluate the usefulness of Doppler echocardiography as a noninvasive alternative to routine endomyocardial biopsies (EMBs) for acute cardiac allograft rejection (ACR) surveillance. The authors found that a first-line routine echocardiographic monitoring of heart transplanted patients allows the replacement of routine EMBs (performed at predefined time intervals) by a reduced number of optimally timed diagnostic EMBs, without any risk for the long-term functional stability of the transplanted heart. These observations are important because routine EMBs are distressing (particularly for children) and potentially risky for patients (tricuspid valve damage, myocardial perforation etc.). Due to differences in the interpretation of EMB specimens mainly because of non-routine screening for antibody-mediated rejection (AMR), sampling errors related to the patchy nature of AR and inter-observer variability the prevalence of 'biopsy-negative' AR (echocardiographic and hemodynamic features suggestive of significant ACR which become reversible by AR therapy) can reach 20% (Miller CA et al. Heart 2013; 99:445-53; Subherwal S. et al. Transplant Proc 2004; 6(10): 3171-72). However, a systematic review and metanalysis (Lu W. et al., PLoS ONE 2015; 10(3): e0121228. revealed that no single conventional ECHO parameter showed a reliable diagnostic performance for ACR. It would be therefore useful to include this meta-analysis into the discussion. It should also be emphasized in the "Discussion" that the value of the detection of diastolic dysfunction in the long-term management of HTx recipients may be diminished by effects of myocardial fibrosis, ventricular hypertrophy, and accelerated cardiac allograft vasculopathy with and also without focal coronary stenoses. Therefore, important heart transplant units rely increasingly more on the tissue Doppler-derived wall motion than on the conventional flow Doppler measurements. In this regard, the radial wall motion monitoring of the left ventricular basal posterior wall in the parasternal long- and/or short-axis views appeared particularly useful. It is also important to emphasize in the "Discussion" that acute changes of echocardiographic parameters are more relevant for ACR diagnosis than their current value, which depends relevantly also on the post-transplant time.

In row 163 even if Desruennes et al. put it this way, "LV hypertrophy with wall thickness >12 mm…" is misleading. In fact sudden increase in LV wall thickness and not LV hypertrophy can be considered as a sign of ACR.

Reply:

Reviewer B highlights relevant points in his comments.

1. In response to the commentary "the prevalence of 'biopsy-negative' AR (echocardiographic and hemodynamic features suggestive of significant ACR which become reversible by AR therapy) can reach 20% », we added the following two references to the revision of our manuscript (lines 9-11, page 14) as suggested by Reviewer B:
    - *Miller CA et al. Heart 2013; 99:445-53;*
    - *Subherwal S. et al. Transplant Proc 2004; 6(10): 3171-72*

2. To answer to the following commentary "However, a systematic review and metanalysis (Lu W. et al., PLoS ONE 2015; 10(3): e0121228.) revealed that no single conventional ECHO parameter showed a reliable diagnostic performance for ACR. It would be therefore useful to include this meta-analysis into the discussion.", we added the suggested reference : *Lu W. et al., PLoS ONE 2015; 10(3): e0121228* (line 2, page 16).

3. Reviewer B highlights the fact that "the detection of diastolic dysfunction in the long-term management of HTx recipients may be diminished by effects of myocardial fibrosis, ventricular hypertrophy, and accelerated cardiac allograft vasculopathy with and also without focal coronary stenosis".

   We agree with Reviewer B that this should be considered for long-term follow-up of HTx recipients as already addressed in the Discussion (lines 19-23, page 13) as follow: *"in part explainable by the high prevalence of relaxation disturbances along with the progressive reduction of myocardial compliance (e.g. high stiffness due to increased fibrosis due to CAV or microcirculatory dysfunction) during years after HT. This limitation can be addressed by analyzing the variations of diastolic parameters over the time for each patient instead of considering each variable independently at a time-point."* We emphasized this point in the revision of our manuscript as follows: "*Tissue-Doppler imaging is increasingly applied by transplant teams as a more accurate assessment than conventional flow-Doppler*."(lines 23-24, page 13).

4. We thank Reviewer B for the following commentary "It is also important to emphasize in the "Discussion" that acute changes of echocardiographic parameters are more relevant for ACR diagnosis than their current value, which depends relevantly also on the post-transplant time."

   Acute changes of ultrasound parameters are considered as diagnostic criteria for ACR in our study as mentioned in the "Material and Methods" section: *"Significant decrease in IVRT and PHT, and/or significant increase in E wave peak velocity, between two consecutive examinations (≥20% changes) in a same patient were also considered as a marker of impaired diastolic function compatible with early rejection" (line 15-17, page 7).*

   As recommended by Reviewer B, we now underscored that point in the revision of the manuscript as follows : "*Last, acute changes in ultrasound variables between two consecutive examinations have also to be considered as a marker of diastolic*

dysfunction and could therefore be related to acute allograft rejection." *(lines 6-8, page 16)*.

5. We fully agree that acute increase of myocardial thickness is more reliable to ACR than "simple" LV hypertrophy. We added *"Acute"* (line 19, page 7) in order to respond to the commentary "*In row 163 even if Desruennes et al. put it this way, "LV hypertrophy with wall thickness >12 mm…" is misleading. In fact sudden increase in LV wall thickness and not LV hypertrophy can be considered as a sign of ACR*".