The Importance of Histopathological Examination for Safety in Heart Valve Transplantation – Evaluation of Histopathological Findings in Heart Tissues from Valve Donors

Wee Ling Heng1*, Siang Hui Lai2, Yeong Phang Lim1 and Chong Hee Lim1
1National Cardiovascular Homograft Bank, Department of Cardiothoracic Surgery, National Heart Centre Singapore
2Department of Pathology, Singapore General Hospital, Singapore

Abstract

Most, though not all, heart valve banks performed routine histopathological examination of heart tissues after valve donation. Histopathological examination will enhance the safety of heart valve homografts for transplantation. This is highlighted by a milestone case of cardiac sarcoidosis, a potentially fatal condition which can involve valve leaflet, in a live donor. Since then, testing is mandated for all our bank’s donors, with the objective of detecting any cardiac-related contraindications, which might be missed during donor screening. It is also a valuable tool in providing additional information about the cardiac cause of death in deceased donors, especially those who passed away suddenly or from unknown cause. A review of histopathological findings in our donors’ heart tissues revealed numerous pathological features despite the small sample size (n=50). Of the deceased donors, 62.9% were diagnosed with pathological features, among which 72.7% had multiple abnormalities. 30% of our bank’s donors were heart recipients, who had multiple cardiac abnormalities. Some pathological findings were found to be interrelated. A donor heart with cardiomegaly revealed no pathological features, emphasizing the necessity to correlate pathological results with clinical data collated during donor screening for a comprehensive clinical picture in the determination of tissue suitability.

Keywords: Cardiac valve donation; Heart; Pathology; Tissue donation; Transplantation

Introduction

Heart valve homograft remains the preferred graft for children and females of child-bearing age, although its usage accounts for merely a small proportion of heart valve implants in adults [1]. They are especially efficacious for valve replacement in endocarditis and for adult congenital heart patients, who previously underwent valve replacement in childhood. Despite advances in bioprosthetic and mechanical substitutes, the other advantages of homograft which favour its usage includes superior haemodynamic performance over most stented bioprostheses, low incidence of thromboembolic complication, greater resistance to infection and no requirement for anti-coagulation [2].

Currently, the major limitation of homograft use is its limited availability for transplantation, in particular the small valve homografts required for paediatric cases. To expand the donor pool, the National Cardiovascular Homograft Bank (NCHB) recovered heart valves not only from deceased donors, but from live donors, who are the heart recipients as well. When hearts valves are procured from heart recipients, histopathological examination of heart tissue becomes crucial. This is because this group of unique donors has severe heart conditions which might affect the competence and safety of their valves.

The objective of this article is to present a significant case study of a live donor who was diagnosed with cardiac sarcoidosis, and its implication in improving the evaluation process of heart valve suitability in our bank. A review of pathological findings in the heart tissues of all NCHB donors is also presented.

Method

During the first year of NCHB operations in 2008, histopathological examination was performed only on explanted native remnant hearts from live donors. This was a routine procedure for heart transplantation. National Heart Centre Singapore’s (NHCS) heart transplant team would dispatch remnant hearts preserved in 10% buffered formalin to the Singapore General Hospital (SGH) Department of Pathology for histopathological examination. Gross examination of the remnant hearts were conducted, which included documentation of its size, the weights and lengths of individual fragments, evaluation of cardiac chambers, the remaining heart valves, myocardium and coronary arteries. For microscopic examination, tissues were dissected at 5 microns for histological sections and routinely stained with hematoxylin, for subsequent orientation. These specimens were fixed in formalin or placed onto a histosette, with full-thickness myocardium, aorta, and occasionally pulmonary and coronary arteries separation of aortic and pulmonary valves in the NCHB processing laboratory, tissue specimens of the left and right atrial and ventricular myocardium, aorta, and occasionally pulmonary and coronary arteries were dissected. The tissues were either directly preserved in the 10% buffered formalin or placed onto a histosette, with full-thickness sections facing upwards, before immersion in formalin. These specimens subsequently underwent wax embedment. Histological sections were at 5 microns and routinely stained with hematoxylin and eosin, Masson’s Trichrome for connective tissue and van Gieson technique for elastic tissue. The sections were examined by the second author.

From year 2009 onwards, fragments of the heart were routinely dispatched for detailed evaluation by the second author. Prior to the separation of aortic and pulmonary valves in the NCHB processing laboratory, tissue specimens of the left and right atrial and ventricular myocardium, aorta, and occasionally pulmonary and coronary arteries were dissected. The tissues were either directly preserved in the 10% buffered formalin or placed onto a histosette, with full-thickness sections facing upwards, before immersion in formalin. These specimens subsequently underwent wax embedment. Histological sections were at 5 microns and routinely stained with hematoxylin and eosin, Masson’s Trichrome and elastic van Gieson stains. The sections were evaluated

*Corresponding author: Heng Wee Ling, National Cardiovascular Homograft Bank, National Heart Centre, Singapore, Mistri Wing, 17 Third Hospital Avenue, Singapore 168752, Tel: 6436 7577; Fax: 6221 0019; E-mail: heng.wee.ling@nhcs.com.sg

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for the presence of pathological conditions such as cystic medial
degenaration or laminar necrosis of the aorta, myocardial ischamia,
muscular degeneraration and cardiomiyopathy, the presence and extent
of cellular inflammation and other clinically significant pathological
features. A brief summary of the medical history of the donor was also
submitted for review and appropriate clinical-pathological correlation.

**Results**

In the first year of operations, NCHB was not required to perform
histopathological examination on the hearts of deceased donors. A
milestone in further enhancing the safety of homografts occurred
after a routine histopathological examination of a remnant heart
from a live donor revealed granulomatous myocarditis with features
suggestive of cardiac sarcoidosis. The diagnosis of this autoimmune
disease was favoured against that of giant cell myocarditis due to the
transmural involvement and vasculitic process in conjunction with
well-formed granulomas, a prominent T-cell infiltrate and notable
absence of eosinophils within the inflammatory infiltrate. Previously,
the donor was diagnosed with idiopathic cardiomiyopathy and had
multiple episodes of congestive cardiac failure. This contraindication
discovered in the post-processing phase subsequently led to a discard
of the valves. After the discovery of this contraindication in a donor,
NCHB implemented mandatory histopathological examination for all
valve donors.

Histopathological findings presented in Table 1 originated from
deceased donors between 1.5-65 years old. Only 37.1% of the heart
specimens from deceased (brain dead and cardiac death) donors had
normal heart structure upon gross examination with no significant
pathological finding. 8.6% of deceased donors had abnormal
enlargement of the heart, a condition known as cardiomegaly detected
by macroscopic examination. A majority of 62.9% were diagnosed
with cardiac abnormalities, among which 72.7% had multiple
abnormalities. Of the donors with cardiac abnormalities, 54.5% had
myocyte hypertrophy. 36.4% had either mild atheroma or coronary
atherosclerosis, in contrast to live donors who usually had severe
atherosclerosis. While these donors were developing risk factors which
might lead to the onset of heart problems, some donors already had
pre-existing heart diseases: 18.2% had cardiac fibrosis. 9.1% had either
primary or hypertrophic cardiomiyopathy, which were hereditary
cardiac diseases as a result of genetic mutation. 4.5% had features
suggestive of cystic medial degeneration of the aorta. Contraction band
necrosis was observed in another donor heart, probably due to the
traumatic events leading to her demise rather than a pre-existing heart
condition.

From Table 2, the results from live donors aged between 35–59 years
were presented. 30% of heart valve donors were live donors. All of them
had multiple cardiac abnormalities, among which a majority of 60% had
cardiomegaly upon macroscopic examination. Pathological findings
discovered included various kinds of cardiomyopathies in 46.7% of the
live donors, ischaemic heart disease in 20% and cardiac sarcoidosis in
6.6%. 26.7% of the donors developed cardiac fibrosis as a result of
myocardial ischamia. One of the donor hearts became so extensively
fibrotic in the epicardium due to high-grade cellular rejection of his
previous transplanted heart, which upon gross examination at the
recovery site, it was rejected by the Medical Director. 53.3% had severe
coronary atherosclerosis. Allograft vasculopathy affected 13.3% of
donors. 6.6% developed myxoid degeneration of mitral valves.

**Discussion**

The importance of histopathological examination was highlighted
when one of our live donors' remnant hearts was diagnosed with
granulomatous myocarditis of cardiac sarcoidosis. This condition
would easily have evaded detection if it was present in a deceased donor,
as histopathological examination was not mandated for heart tissues of
such donors back in 2008. Should this occurred it might lead to major
clinical and safety consequence to the recipient as transmission of
sarcoidosis from a heart donor to the recipient, though extremely rare,
had been reported in a case of orthotopic heart transplantation [3].

Cardiac sarcoidosis is an uncommon but potentially fatal condition
of unknown etiology. It has been reported to occur in isolation without
evidence of sarcoidosis in other parts of the body. Sarcoid granuloma
may occur in any location of the heart, with the most common site of
involvement being the myocardium [4,5]. However, there had also been
reported cases of valve leaflet involvement, leading to regurgitation [5].
Sudden death is the most severe manifestation of cardiac sarcoidosis,
which usually causes ventricular arrhythmia or complete heart blockage
as a result of extensive myocardial involvement [4]. A study in Japan
revealed that of the sarcoidosis-related deaths, 46.9% was associated
to cardiac sarcoidosis. However, clinical diagnosis had only been
made in 26.7% of the cases [6], revealing the disease's variable clinical
manifestation [5].

Unlike donors with accumulative disorders such as cardiac
amyloidosis, a criterion which resulted in the exclusion of a potential
live donor during initial donor assessment, the diagnosis of cardiac
sarcoidosis in a live patient is more challenging. This is because
endomyocardial biopsy, which is considered a gold standard for its
detection, is still a relatively insensitive test yielding low diagnostic rate.
While cardiac amyloidosis reveals diffused myocardial involvement
throughout the myocardium [7], sarcoid granuloma shows localised
or heterogeneous distributions within the myocardium and other parts
of the heart [4,5,7]. Only 25% of the patients with cardiomiyopathy
and clinical diagnosis of sarcoidosis were found to have non-

| Types of Pathological Findings                                      | Number (N=15) |
|--------------------------------------------------------------------|---------------|
| Severe coronary atherosclerosis                                   | 8             |
| Ischaemic heart disease                                           | 3             |
| Dilated cardiomyopathy                                            | 4             |
| Cardiac fibrosis                                                  | 4             |
| Chronic ischaemic cardiomyopathy                                  | 2             |
| Allograft vasculopathy                                            | 2             |
| Post-myocardic cardiomyopathy                                     | 1             |
| Myxoid degeneration of mitral valves                              | 1             |
| Cardiac sarcoidosis (Contraindication)                            | 1             |

Table 2: Significant cardiac abnormalities in live donors.

Table 1: Significant cardiac abnormalities in deceased donors.
caseating granulomas on endomyocardial biopsy [5]. Moreover, its symptoms, such as an abnormal electrocardiogram, arrhythmia, valvular abnormalities and dilated cardiomyopathy were non-specific for its diagnosis [4,5]. This was probably the case why the live donor developed cardiomyopathy of unknown etiology, leading to cardiac failure requiring transplantation. It was only after her native heart remnant was comprehensively studied by histopathological procedure that her condition was fully elucidated.

After this experience, we realise the importance of histopathological examination and mandate it to become a routine test for all donors since 2009. The objective is to detect any cardiac-related contraindications which might otherwise be missed during donor screening. Besides, it is a valuable tool in providing additional information about the cardiac cause of death in deceased donors, especially those who passed away suddenly or from unknown cause. Although our bank had not encountered donor referral of sudden cardiac deaths due to non-atheromatous or undiscernable cause, there is a possibility of donors who has morphologically normal hearts with microscopic pathological features yet to be identified, during initial donor screening. Although some diagnoses might be missed due to removal of valves from remnant hearts, it was shown that valve donation did not significantly impair the outcome of the examination [8]. Usually, macroscopically normal hearts would reveal microscopic abnormalities. For instance, an Italian study reported that among the 28% of their macroscopically normal hearts examined, histological examination disclosed that 79% of the hearts contained pathological substrates [9]. In another French study, 10% of unexplained sudden deaths had pathological evidence of arrhythmogenic right ventricular cardiomyopathy [10]. Histopathological examination might also reveal the presence of other cardiac abnormalities, infections, malignancies, systemic or connective diseases which might be relevant for heart valve transplantation [11].

Many pathological features were discovered despite the small sample size (n=50). This was a significant finding, which further emphasised the importance of histopathological examination in macroscopically normal hearts [12]. However, as heart valves were determined to be unaffected by most of these cardiac abnormalities and the conditions were unlikely to be transmissible to recipients (with the possible exception of cardiac sarcoidosis), they were not contraindications for valve donation. Nevertheless, they provided important reference to the overall condition and function of the donor hearts.

Some pathological findings were discovered to be interrelated. For instance, the presence of myocyte hypertrophy represents a physiological adaptive response to a rise in blood pressure [13]. Hence, its presence is likely to suggest hypertensive changes in the myocardium, which in turn might be an outcome for coronary atherosclerosis. In another instance, severe coronary atherosclerosis had led to myocyte death, myocardial infarction, subsequent scarring or fibrosis, and finally chronic ischaemic cardiomyopathy. This was presented in a case of a live donor diagnosed with severe coronary atherosclerosis, which progressed to end-stage cardiac failure as a result of chronic ischaemic cardiomyopathy, the end-point to myocardial ischaemia.

Besides coronary atherosclerosis, cardiac fibrosis was also found to be generally associated with myocyte hypertrophy. Fibrosis is an expression of interstitial heart disease. For most cases, the endocardium, epicardium and myocardium were the more common sites of involvement. The abnormal proliferation of cardiac fibroblasts and deposition of fibrillar collagen increased diastolic myocardial stiffness and predisposition to heart failure. Although cardiac hypertrophy is a risk factor to cardiovascular disease and increases cardiovascular mortality [13], the development of fibrosis probably has more clinical significance and represents a progression to diastolic cardiac dysfunction. This is because evidence has revealed that the disease is not caused by the mass of myocardium created by hypertrophied myocytes, but rather by the remaining myocardium that is altered by the accumulation of fibrous tissue [14].

It was also interesting to note that of the three deceased donors' hearts which had cardiomegaly, one revealed no microscopic pathological feature. This might indicate a limitation of our bank's practice, which prevented a complete pathological diagnosis of the heart remnant. This was because local legislation allowed for the recovery of heart valve block, whereas the heart remnants had to be returned to the donor's thoracic cavity. Therefore, only representative tissue specimens could be dispatched for examination, which might be inadequate for detection of all clinical abnormalities. This further emphasised the necessity to correlate histopathological results of the tissues with clinical data collated during donor assessment for a more comprehensive clinical picture in the determination of tissue suitability.

Although most banks routinely performed histopathological examination on heart tissues after valve donation, there are some banks which do not practise it [11]. As histopathological examination will definitely enhance the safety of heart valve homografts for transplantation, we strongly recommend all heart valve banks to consider performing this procedure for the benefit of their recipients.

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