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Case report

# Epithelial to mesenchymal transition in endomyocardial biopsies from orthotopic heart transplant recipients

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## SUMMARY

Epithelial to mesenchymal transition (EMT) occurs when cells lose morphological features of epithelial cells, such as cell-to-cell adhesion, and gain features of mesenchymal cells, including elongation and flattening. These cells also lose expression of epithelial immunohistochemical markers. In this report, we present a 55-year-old Caucasian male patient who underwent orthotopic heart transplant and immunosuppressant therapy with tacrolimus and mycophenolic acid. Seven and a half months later, an endomyocardial biopsy revealed a hypercellular, atypical lesion. Evaluation was negative for acute cellular rejection and post-transplant lymphoproliferative disorder. Histopathologic features and immunohistochemical stains were consistent with EMT. We subsequently identified four additional cases of EMT in patients who underwent orthotopic heart transplantation and received a similar immune suppression regimen. EMTs have been reported to occur in lung and kidney allografts; however, this is the first known report describing this entity in a heart transplant recipient.

## BACKGROUND

Heart transplantation provides a valuable therapeutic option for patients suffering from heart failures unresponsive to medical therapy or with life-threatening arrhythmias. However, these patients are at high risk of organ rejection and require close monitoring with frequent endomyocardial biopsies (EMBs), especially during their first post-transplant year. Patients take immunosuppressants to prevent organ rejection and are routinely evaluated with surveillance biopsies once per week for several months and then every 6–8 weeks between 3 and 12 months.<sup>1</sup> The EMB frequency then reduces to quarterly, biannually or annually.<sup>1</sup> Biopsies from heart transplant recipients may sometimes show features erroneously resembling acute cellular rejection (ACR), such as previous biopsy sites or Quilty effects.<sup>2</sup> Therefore, it is critical to understand how to differentiate between true ACR and conditions resembling ACR to avoid incorrectly diagnosing a patient with allograft rejection.

Previous biopsy sites are a common histological finding that can resemble ACR: they are observed in approximately 16% of EMBs in the postoperative period.<sup>2</sup> A recent biopsy site lesion may exhibit

granulation tissue, focal surface fibrin deposition, interstitial haemorrhage, coagulative occasional necrosis of myocytes, a mixed mononuclear and polymorphonuclear infiltrate and karyorrhectic debris. Later biopsy sites will often show variable fibrosis, mononuclear cell infiltrates and hemosiderin-laden macrophages.<sup>2</sup> Another possible finding in post-transplant patient biopsies is infections, including cytomegalovirus (CMV) and toxoplasmosis. CMV can be distinguished from ACR by the viral inclusions in the myocytes and the presence of frequent neutrophils compared with the typical inflammatory infiltrates associated with rejection.<sup>2</sup> Toxoplasmosis is identified based on the toxoplasma cyst visualised in the myocytes.<sup>2</sup> Another finding is post-transplant lymphoproliferative disorders (PTLD) which are usually distinguished from ACR by immunohistochemical staining of CD20 +B cells. If Epstein-Barr virus (EBV) is identified in the lymphocytic infiltrate, it aids in the diagnosis of PTLD.<sup>2</sup>

A unique finding that has not been described in the literature and may be observed in EMB from heart transplant recipients is epithelial to mesenchymal transition (EMT), a reparative process mediated by transforming growth factor beta-1 (TGF- $\beta$ 1), a profibrotic agent. EMTs have been identified in lung and kidney transplant recipients and in lung transplant patients receiving immunosuppressants such as cyclosporine, azathioprine, mycophenolic acid and sirolimus.<sup>3</sup> Here, we describe a case of a patient who received a heart allograft and a post-transplantation EMB revealed a hypercellular, atypical lesion that after thorough work-up and evaluation, appears to be consistent with EMT.

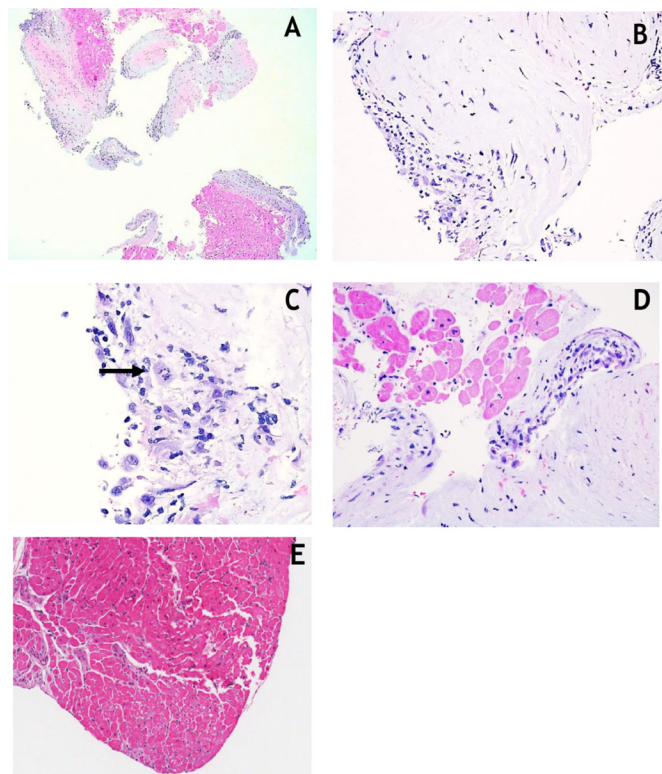
## CASE PRESENTATION

A 55-year-old Caucasian male patient with a medical history of atrial fibrillation, ventricular tachycardia, non-ischaemic cardiomyopathy, heart failure with reduced ejection fraction (EF=15%–20%) and chronic kidney disease stage III, was admitted to our institution for orthotopic heart transplantation. The patient was induced with the following immunosuppressant agents: thymoglobulin (150mg), mycophenolate mofetil (MMF) (500mg) and methylprednisolone (500mg). Right heart EMBs were conducted weekly for the first 8 weeks and



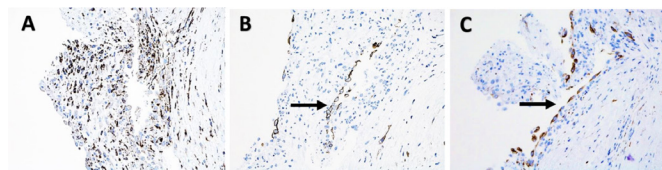
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**Figure 1** (A–D) H&E stains of this patient’s endomyocardial biopsy (EMB) at 7.5 months post-orthotopic heart transplant showing a hypercellular, atypical endocardial lesion that was composed of enlarged, atypical-appearing, pleomorphic cells. Focal mitotic figures were also seen (C, arrow). (E) Representative image of an EMB from another patient that was graded as grade 0R (International Society for Heart and Lung Transplantation (ISHLT)) and did not contain any evidence of epithelial to mesenchymal transition .

then every 3 months thereafter. These biopsies were negative for evidence of transplant rejection. Five months later, the patient was admitted complaining of weight loss, anorexia and loose stools and was diagnosed with severe colitis secondary to MMF. During this admission, the patient’s immunosuppression regimen was modified to include reduction in tacrolimus and valcyte dosage; his MMF formulation was changed to myfortic (mycophenolic acid). After approximately seven and half months post-transplantation, one of his EMBs revealed a hypercellular, atypical endocardial lesion composed of enlarged, pleomorphic cells (figure 1).



**Figure 2** (A–C) Endomyocardial biopsy immunohistochemical stains. The CD68 stain highlighted abundant macrophages scattered throughout the lesion (A). CD34 positive cells were also observed in the same location, consistent with the transition of an epithelial phenotype to a more mesenchymal phenotype (B, arrow), and a Pan-cytokeratin (AE 1/3) stain highlighted positive cells lining the endocardial surface of the lesion (C, arrow).

## INVESTIGATIONS

His EMB did not show any inflammation within the cardiac myocytes or the interstitium. However, there was an atypical appearing inflammatory infiltrate associated with the endocardial surface. We further evaluated the patient’s clinical history and analysed the EMB with additional stains including CD68 (figure 2A), CD34 (figure 2B), CK AE1/3 (figure 2C), CD3, PAX-5, C4d, CD20, kappa, lambda, WT1, adenovirus and EBV (EBER-*ish*) to determine the aetiology of the lesions. There was evidence of EMT on the outer surface of the biopsy based on the morphological appearance and the CD34 and pancytokeratin (AE1/3) immunostains were positive along cells lining the endocardium (figure 2). The CD68 immunostain highlighted abundant macrophages attached to the endocardial surface and scattered throughout the lesion. CD3 highlighted focal lymphocytes. PAX-5 (B-cell-specific activator protein), C4d, CD20, kappa and lambda light chain and WT1 immunostains were negative (images not shown).

## DIFFERENTIAL DIAGNOSIS

Initially, we were concerned that the patient may have developed PTLD based on the atypical appearance of the cells within the lesion. PLTD may be diagnosed by immunophenotyping to confirm the presence of B-cells, plasma cells with polytypic light chains and in situ hybridisation to show evidence of EBV.<sup>4</sup> Therefore, we performed staining against kappa and lambda light chains, CD20 and in situ hybridisation of EBV (EBER), as mentioned in the investigation section. There was no evidence of a lymphoproliferative disorder and importantly, stains for EBV were negative. Stain for adenovirus was also negative. However, there was evidence of EMT on the outer surface of the specimen. The phenotypical appearance of the lesion was a hypercellular, atypical endocardial lesion composed of enlarged and pleomorphic cells. The immunohistochemical stains showed that the atypical-appearing cells were forming a new layer on the endocardial surface and were transitioning from epithelial-like (AE1/3+) to mesenchymal-like (CD34+) and were thus undergoing EMT. These features are consistent with a reparative process occurring along the endocardial surface, likely due to either the patient’s recent transplant procedure or previous EMBs.

## TREATMENT

No treatment was provided for EMT for this patient. The patient remains compliant with his post-cardiac transplantation immunosuppression medications: mycophenolic acid 250 mg capsules per-oral daily, tacrolimus 0.5 mg capsule per-oral every Monday, Wednesday, Friday and Saturday in the evening, and prednisone 5 mg tablet per-oral daily.

## OUTCOME AND FOLLOW-UP

This patient has been routinely having surveillance EMBs and all have remained negative for rejection. As per his latest transthoracic echocardiogram, the patient has normal left ventricular function with EF of 55%–60%, right ventricular dilatation and dysfunction with pressure/volume overload, pulmonary hypertension, and moderate tricuspid and mitral valve regurgitation.

We subsequently identified four additional EMT cases, in patients who had also undergone orthotopic heart transplantation and received a similar immune suppression regimen. The additional four patients had similar EMB findings; their subsequent biopsies showed similar lesions, although slightly less cellular, with increased fibrosis. Graft function remained normal in all patients.

## DISCUSSION

Epithelial cells are distinguished from mesenchymal cells based on histological features. In epithelia, cells are closely associated and adhering tightly to serve as a protective barrier and absorptive/secretory surface.<sup>5</sup> In occurrence of injury, epithelia often respond by repair, necrosis or apoptosis. Another alternative reparative process is EMT. EMT has been defined as epithelial cells acquiring the features and motility of mesenchymal cells.<sup>6</sup>

EMT is divided into three different subtypes: type 1—EMT that occurs in the embryo where epithelial cells transit to mesenchymal cells and migrate to create new organs, type 2—EMT that is involved in wound repair and tissue regeneration and organ fibrosis and type 3—EMT that occurs in epithelial carcinomas.<sup>7</sup> In this patient, type 2 EMT is most likely. In type 2 EMT, epithelial cells lose their epithelial cell properties and acquire spindle-cell shape accompanied by secretion of extracellular matrix reduction of epithelial markers, and an increase in mesenchymal markers: matrix metalloproteinases,<sup>8</sup> vimentin, N-cadherin, fibroblast-specific-protein-1 and  $\alpha$ -smooth muscle actin ( $\alpha$ -SMA).<sup>7</sup>

After injury, cells go through a course of events starting with acute inflammation and ending with wound healing/fibrosis and scar formation. During the wound healing phase, TGF- $\beta$ , a profibrotic factor, is released which is believed to be the main inducer of EMT in epithelial cells. There are also other physical factors that can lead to EMT such as: hypoxia, high glucose levels,<sup>9 10</sup> albumin,<sup>9 10</sup> angiotensin II,<sup>9 10</sup> inflammatory mediators<sup>11</sup> such as tumour necrosis factor  $\alpha$  (TNF- $\alpha$ ) and matricellular proteins.<sup>7</sup> These factors can induce the phenotypical changes seen in epithelial cells and the transition to the mesenchymal phenotype.

We hypothesise that this patient developed an EMT as part of a reparative process due to his recent transplant procedure and multiple post-transplant EMBs. As the site of injury is trying to heal, TGF- $\beta$ 1 may be released which activates Smad2 and Smad3 (signal transducers for TGF- $\beta$  receptor) through phosphorylating the C-terminal by the T $\beta$ RI (TGF- $\beta$  receptor); this leads the binding of Smad4 and the formation of a trimer which translocate into the nucleus and regulate gene transcription.<sup>12</sup> This process results in the regression of the epithelial markers gene expression and the activation of the mesenchymal gene expression thus resulting in EMT.<sup>12</sup> It has also been noted that TNF- $\alpha$ , produced by macrophages, dramatically accentuates the phenotypical appearance and expression of mesenchymal genes.<sup>10 13</sup>

EMT found on biopsy may be concerning initially, however, it is an important process to consider since long term accumulation of fibrosis, caused by the transition from epithelia to mesenchymal tissue, can lead to permanent scarring and cause impairment of the organ function.<sup>7</sup> To our knowledge, EMT was not previously identified post-heart transplantation. However, it has been a cause of airway remodelling after lung transplant and deterioration of kidney transplants. It has been noted that post-lung transplant, many patients develop bronchiolitis obliterans, and based on immunobiology chemistry, a reduction in epithelial markers and an increase in mesenchymal markers: vimentin and  $\alpha$ -SMA were identified indicating a transition from epithelium to mesenchymal cell type.<sup>8 14</sup> In kidney transplantation, biopsies from deteriorating allograft kidneys with impaired functions showed a reduction in epithelial markers and an increase in mesenchymal markers.<sup>15</sup>

To determine the contributing factors for EMT-related bronchiolitis obliterans after lung transplantation, immunosuppressive therapy regimens were evaluated to see if they contributed to the fibroproliferative transformation. Results of the study

revealed that treatment with cyclosporine, azathioprine, mycophenolic acid and sirolimus resulted in an increase in TGF- $\beta$  and morphological changes consistent with transition of epithelial cells to the mesenchymal cell type.<sup>3</sup> Similarly, another study showed that immunosuppressive therapy contributes to renal tubular EMT.<sup>3</sup> However, no research has been performed to show the effect of immunosuppressive agents on EMT in patients post-heart transplantation. Based on previous studies, the accumulation of fibrosis can result in organ dysfunction. It has been suggested that potential areas of investigation should involve hepatocyte growth factor or bone morphogenetic protein-7, for blocking TGF- $\beta$ /Smad signalling pathway for EMT regulation thus slowing the progression of EMT.<sup>7 16</sup>

In this patient, many contributing factors may have caused the development of EMT; he was status-post orthotopic heart transplantation and he underwent multiple EMBs, which may have resulted in EMT as a reparative process post-injury at the biopsy site. The patient has also been taking mycophenolic acid, an immune suppressive agent that has been shown to lead to EMT in transplanted lungs. In summary, the main causative agent for this patient's EMT is still not clear. However, the process is likely to be reparative.

## Learning points

- ▶ Epithelial to mesenchymal transition (EMT) is involved in wound repair, tissue regeneration and organ fibrosis. It can occur at biopsy sites due to injury caused by obtaining biopsy.
- ▶ Immunohistochemistry should be performed to exclude the possibility of other causes of the lesion such as post-transplant lymphoproliferative disorders or underlying infection.
- ▶ Transforming growth factor beta (TGF- $\beta$ ) is the main inducer of EMT and its effect it is further accentuated by tumor necrosis factor  $\alpha$ , produced by macrophages. In other types of solid organ allografts (eg, kidney and lung), reduction of inflammation can slow the progression of EMTs, and blocking of TGF- $\beta$  effects may be explored as a treatment option for EMT in the future.
- ▶ Overtime, fibrosis accumulation of EMT can result in scar formation and organ dysfunction has been described in other types of solid organ transplants, although the significance in heart transplant recipients is currently not known.

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