

ORIGINAL PAPER

PREVALENCE OF THE QUILTY EFFECT IN ENDOMYOCARDIAL BIOPSY OF PATIENTS AFTER HEART TRANSPLANTATION – FROM CELLULAR REJECTION TO ANTIBODY-MEDIATED REJECTION?

SYLWIA SZYMANSKA¹, WIESŁAWA GRAJKOWSKA¹, MALGORZATA SOBIESZCZANSKA-MALEK², TOMASZ ZIELINSKI², MICHAŁ PYZŁAK^{1,3}, MACIEJ PRONICKI¹

¹Department of Pathology, The Children's Memorial Health Institute, Warsaw, Poland

²Department of Heart Failure and Transplantation, The Institute of Cardiology, Warsaw, Poland

³Department of General and Experimental Pathology, Medical University of Warsaw, Warsaw, Poland

The significance of the Quilty effect (QE) is not fully understood. It was once proposed to be related to acute cellular rejection (ACR). We aim to assess the relation between QE prevalence and antibody-mediated rejection (AMR).

One thousand three hundred and fifty endomyocardial biopsies (EMBs) from 212 patients who underwent heart transplantation in the years 2001-2013 and survived a period of 30 days after the operation were diagnosed. In all EMBs routine HE staining and additional immunohistochemical staining with polyclonal antibody against C4d were performed. Microscopic findings were classified according to the new ISHLT 2013 criteria. Patients were separated into two groups: group 1 included those with at least one pAMR1 I+ and/or pAMR2 EMB (n = 16), and group 2 included the rest of the patients (n = 196). Presence of QE with distinguishing subtypes A and B (according to the first ISHLT 1990 criteria) was assessed.

One hundred and twenty one EMBs from group 1 and 1229 EMBs from group 2 were diagnosed. Quilty effect type A was found in 16 (13.2%) EMBs in group 1 and in 96 (7.8%) EMBs in group 2, $p < 0.001$. Quilty effect type B was diagnosed in 52 (43%) EMBs in group 1 and in 245 (20%) EMBs in group 2, $p < 0.001$. The QE was not present in 53 (43.8%) EMBs in group 1 and in 888 (72.2%) EMBs in group 2, $p < 0.001$.

The relation between QE prevalence and AMR is possible as the QE is present statistically more often in EMBs of patients with C4d capillary depositions.

Key words: heart transplantation, antibody-mediated rejection, Quilty effect, C4d.

Introduction

The Quilty effect (QE) was first described by Billingham in 1981. It was defined as a lymphocytic infiltrate bulging in the endocardium of a cardiac allograft with or without involvement of the myocardium [1]. The clinical significance and mechanism of this lesion are still controversial. Quilty effect inci-

dence in endomyocardial biopsies (EMBs) is also not established – according to some authors it varies from 10% to 20% [2]. The first International Society for Heart and Lung Transplantation (ISHLT) criteria for reporting heart allograft rejection (ISHLT 1990) [3] classified the QE into two subtypes based on its relation to myocardium. Subtype A was supposed to be

limited to the endocardium while subtype B involved the myocardium. Later on, the clinical significance of distinct types was questioned and the new ISHLT 2004 criteria did not sustain the QE classification [4]. In the early 1990s the QE was believed to be related to cyclosporine therapy [5]. A drug toxic effect or immunologic reaction to this immunomodulator was proposed as a potential trigger. Further studies [6, 7] did not prove that hypothesis as the lesion had been found in EMBs of patients treated with different kinds of immunomodulators. Other authors [8, 9] believed that the QE was a kind of low-grade cellular rejection (ACR), specially type B. The potential mechanism was separation of inflammatory infiltrates from the endocardium. Nowadays, this relation seems unlikely, yet some studies in recent years have shown a greater possibility of developing rejection in patients who manifest the QE [2]. Recent studies showed that IL-10 gene polymorphism, associated with decreased cytokine production, was increased in patients with Quilty lesions [10]. It was also proved that the QE had features of a tertiary lymphoid tissue [11]. Most recently, QE areas were reported to be frequently associated with endocardial C4d deposition [12]. C4d is a well-known immunohistochemical marker of antibody-mediated rejection (AMR). Humoral rejection remains a significant factor of unfavorable allograft function and survival. Therefore, the aim of this study was to assess the relation between QE prevalence and AMR.

Material and methods

One thousand three hundred and fifty EMBs from 212 patients who underwent heart transplantation in the years 2001-2013 and survived a period of 30 days after the operation were enrolled in the study. All EMBs were retrospectively examined in the Department of Pathology, The Children’s Memorial Health Institute, by three qualified pathologists unaware of the clinical data. Endomyocardial specimens were taken from the right ventricle, fixed in 4% formalin and embedded in paraffin. The paraffin blocks were cut into 4 μm slices and routinely stained with hematoxylin/eosin (HE). Immunohistochemical (IHC) staining with polyclonal antibodies against C4d (Biomedica Gruppe, 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% of vessels involved). The staining of venular, arterial, or arteriolar endothelial cells, arterial elastic lamina, and the capillaries in the QE were not considered to be indicative of AMR. CD68 stain was not performed in our department. Microscopic findings were classified according to the new ISHLT 2013 classification. Patients were divided into two groups based on the C4d reaction. Group 1 included those with at least one pAMR1 I+ and/or pAMR2 EMB (n = 16), and group 2 included the rest of the patients (n = 196). Presence of QE with distinguishing subtypes A and B (according to the first ISHLT 1990 criteria) was assessed. Information concerning donor-specific antibody (DSA) presence was not available for all patients. For statistical analysis of the result Statistica software was used and the χ² test was applied.

Results

One hundred and twenty one EMBs from 16 patients from group 1 and 1229 EMBs from 196 patients from group 2 were diagnosed. In group 1 pAMR1I+ was present in 12 EMBs (9.9%) and pAMR2 in 11 EMBs (9.1%). Quilty effect type A was found in 16 (13.2%) EMBs in group 1 and in 96 (7.8%) EMBs in group 2, p < 0.001. Quilty effect type B was diagnosed in 52 (43%) EMBs in group 1 and in 245 (20%) EMBs in group 2, p < 0.001. The QE was not present in 53 (43.8%) EMBs in group 1 and in 888 (72.2%) EMBs in group 2, p < 0.001. There was no difference in the frequency of each QE subtype between groups in AMR-positive biopsies. Results are summed up in Table I.

Discussion

The significance and clinical implications of the QE are still not entirely clear although 34 years have passed since the first description of the lesion. The greatest literature impact on the QE comes from the 1990s, when its inflammatory composition and potential mechanism were studied. Some authors connected the QE with cyclosporine therapy [5], the QE being thought to be a kind of ACR [8, 9]. Most of these findings are no longer sustained. In EMBs of patients treated with modern immunomodulators the QE is found as frequently as on previous therapy.

Table I. Prevalence of Quilty effect in patients with and without C4d positivity

QUILTY EFFECT (QE)	C4D (+) EMBs (N = 121)	C4D (-) EMBs (N = 1229)
Any subtype	68 (56.2%) EMBs	341 (27.8%) EMBs
QE type A	16 (13.2%) EMBs	96 (7.8%) EMBs
QE type B	52 (43%) EMBs	245 (20%) EMBs

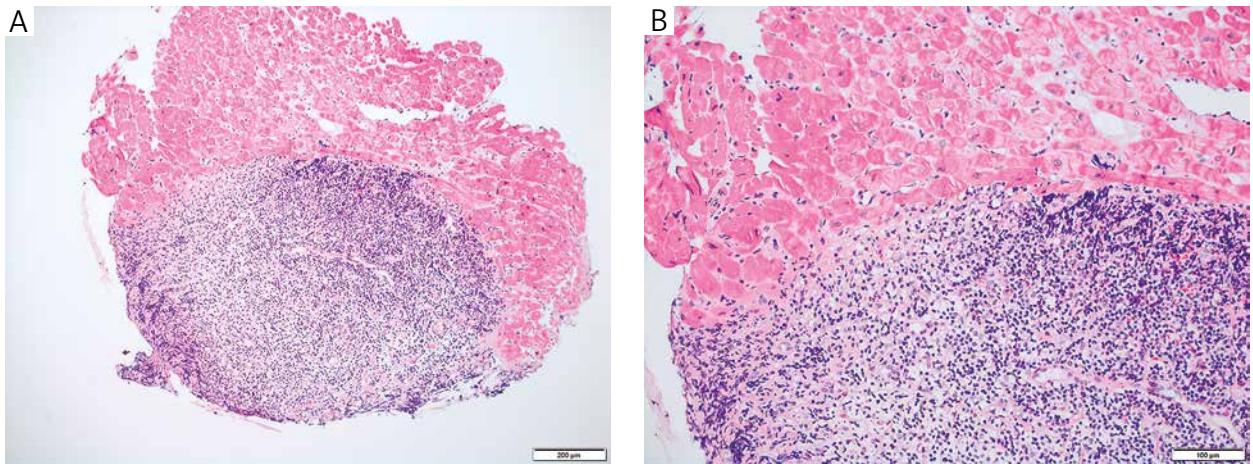


Fig. 1. Quilty effect type A. In the image extensive lymphocytic infiltrate bulging in the endocardium is present. Although the lesion is wide the myocardium is not involved. A) HE staining, original magnification 200×; B) HE staining, original magnification 400×

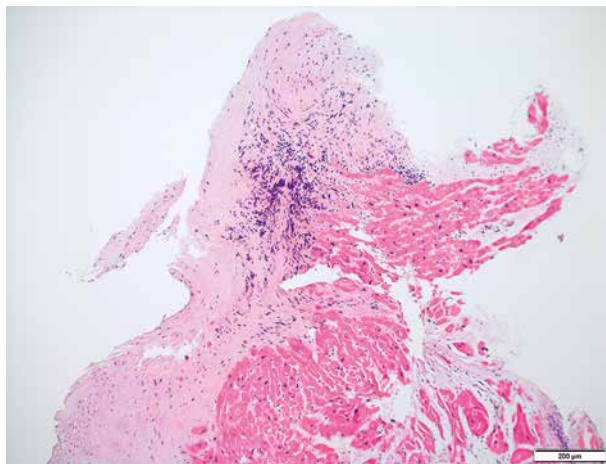


Fig. 2. Quilty effect type B. Lymphocytic infiltrate is moderate but it involves the myocardium. Prominent endocardial fibrosis is also present. HE staining, original magnification 200×

The relation to ACR is also undermined, although a recent study of Zakliczynski *et al.* [13] proved a correlation between QE and ACR, but not between QE and coronary artery vasculopathy (CAV). The authors concluded that the QE seemed to be a marker of the same increased immune system activity that can lead to ACR. The results of our study do not support that finding. First of all, in EMBs diagnosed in our department the QE of any subtype was present more frequently (in 30.3% EMBs) than its average literature incidence. Statistical analysis proved that both QE type A (Fig. 1 A and B) and B (Fig. 2) were found significantly more often in biopsies of patients with at least one C4d-positive staining (Fig. 3), which is a well-known immunohistochemical marker of AMR. We decided to divide the QE into types A and B according to the first ISHLT 1990 criteria, although it is no longer standard practice to verify

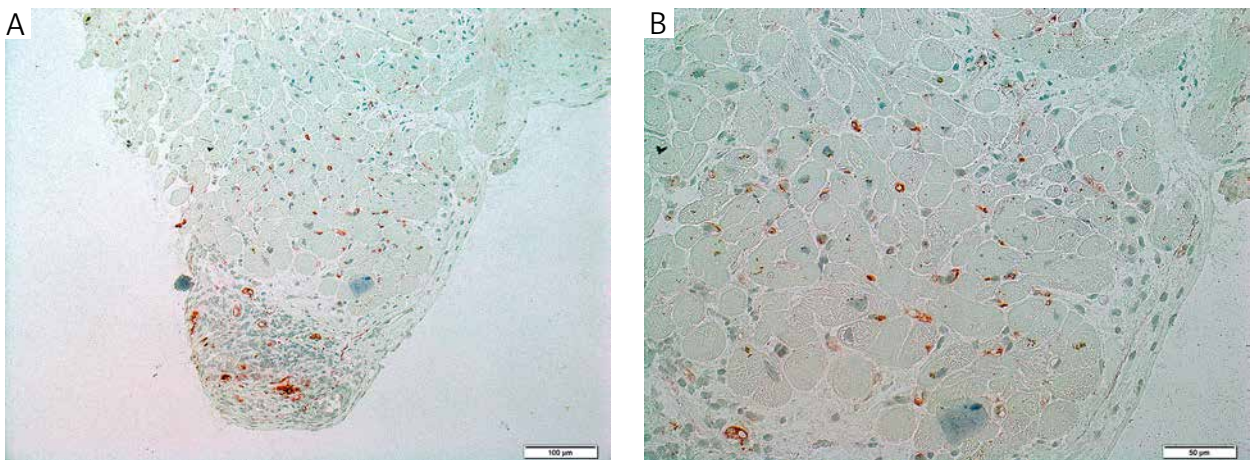


Fig. 3. C4d positive staining in EMB of patient with Quilty effect: A) original magnification 200×; B) original magnification 400×

whether there is any difference in terms of humoral rejection. No difference between the subtypes was found. The obtained results proved that QE classification is justifiable neither from a clinical nor a histopathological point of view. For analysis we chose the chi-squared test because it is the most appropriate tool to present independent normally distributed data. Our study was a retrospective one and initial microscopic diagnosis was present in previous ISHLT criteria; therefore we reclassified all EMBs according to the new ISHLT 2013 criteria. Significance of histopathological changes in terms of humoral rejection is still discussed. Our preliminary data [14] proved that microscopic features suggestive of AMR are found relatively often in EMBs of patients after heart transplantation, regardless of C4d positive stain, and we would like to discuss that matter in a separate paper. In this study we focused on complement depositions in capillaries, as they are believed to be strongly connected with AMR. Therefore we isolated patients who have had at least one EMB with diagnosis of pAMR1 I+ and/or pAMR2. The examination proved that QE was observed statistically more frequently in that group than in the rest of the patients. Concomitant presence of QE and C4d deposition was found in 10/121 EMBs (8.3%), but lesions were often observed in both previous and subsequent biopsies of those patients. Thus, we concluded that a common mechanism of QE and complement deposition development may exist. Nonetheless, the correlation remains rather weak, which may suggest a multifactorial rather than a simple direct relationship. Cano *et al.* [12] published similar observations, but their background was different than ours. They analyzed the C4d-positive linear staining in the endocardium surrounded by the lymphocytes forming the QE lesion. In some cases, the linear C4d deposition extended to the endocardium surrounding the QE area. In their material there were no signs of rejection. In our department, we also sometimes observe linear C4d positivity in the endocardium, but we interpret it as non-specific staining. Cano *et al.* suggested that the concomitant presence of QE and C4d depositions in an allograft heart biopsy, without evidence of rejection, indicated better adaptation of the allograft to the host (“accommodation”) [12]. Our experience supports that hypothesis – most of the C4d-positive patients have never presented any clinical symptoms. Preliminary clinical analysis proved that there were no statistically significant differences in survival between patients with positive staining and without C4d+ staining when Kaplan-Meier survival curves were compared. Obviously, there is still a lot of controversy on the issue. We do not fully understand the mechanism of AMR itself, so it is difficult to propose a common pathogenic pathway between humoral rejection and QE. Most likely, different factors, which

should be further explored, induce coexistence of Quilty lesions and AMR. A major limitation of our study is the lack of DSA detection in all suspected AMR patients. It may be explained by the retrospective character of the study. Most EMBs were archival, diagnosed before 2004. Unfortunately, serum was not preserved in any case and we could not perform the tests. Besides, DSA testing is relatively expensive, and it is not possible to verify it at all times. Nonetheless, the relation between Quilty effect prevalence and AMR may be possible. To the best of our knowledge, our study is the second one to imply a relation between C4d deposition and the QE. Further studies on larger groups of patients are mandatory.

Conclusions

The relation between QE prevalence and AMR is possible as the QE is present statistically more often in EMBs of patients with C4d-positive staining.

The authors declare no conflict of interest.

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Address for correspondence

Sylwia Szymanska
Department of Pathology
The Children's Memorial Health Institute
Al. Dzieci Polskich 20
04-730 Warsaw, Poland
tel. +48 22 815 19 60
fax +48 22 815 19 75
e-mail: s.szymanska@ipczd.pl