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Relationship between CMV and graft rejection after heart transplantation

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Abstract This study, which included 153 heart transplant patients, was designed to determine whether the cytomegalovirus (CMV) status of both donor and recipient may influence graft rejection. The follow-up was 1 year and they all received the same triple-drug immunosuppressive regimen with induction (antilymphocyte serum). There was no difference in the total rejection rate, but an increase in repeated rejection rate was shown in transplant recipients with hearts from CMV seropositive donors ($P < 0.05$). These data strongly suggest the impact of CMV in enhancement but not in induction of rejection. To prevent iterative rejection in the CMV seropositive donor group, antiviral therapy could be proposed during enhancement of antirejection therapy.

Key words CMV · Graft rejection · Heart transplantation · Antiviral therapy

Introduction

Cytomegalovirus (CMV) is a common infectious agent after heart transplantation and virological signs (viremia, antigenemia) of CMV are frequent during graft rejection, but no study has shown a strong relationship between CMV infection and rejection. The latent state of CMV within the endothelium could lead to a focal reactivation during inflammation and, especially, graft rejection without CMV infection or disease. Focal CMV activation could act as an enhancer of graft rejection leading to repeated rejection and increase in immunosuppressive drugs.

Materials and methods

From 1 January 1989 to 1 January 1994, 153 patients were followed after heart transplantation. These patients were alive 1 month after transplantation and they all received the same immunosuppressive protocol. This protocol consisted of corticosteroids 0.2 mg/kg per day, azathioprine 0.5 mg/kg per day and cyclosporine, according to a first year blood level, between 200 and 300 ng/ml. A 5-day course induction was applied with antilymphocyte serum. Virological screening of both donor and recipient was done twice by IgG serology (ELISA); IgG-positive recipient was called R+, IgG-negative recipient was called R–, IgG-positive donor was called D+, and IgG-negative donor was called D–. According to this screening, four groups of patients have been established: group I, R–/D–; group II, R–/D+; group III, R+/D–; and group IV, R+/D+. Graft rejection was detected by clinical signs, echographical data, and confirmed by endomyocardial biopsy. The biopsies were scheduled as follows: each week for the first 2 months, every 2 weeks for the next 4 months, and then monthly until the end of the first year.

International Heart Transplantation Society Classification [1] was used in order to grade the graft rejection. Positive biopsies per patient during 1 year were classified into two groups: untreated grade IA and treated rejection grade IB or greater. We recorded patients with lethal rejection, repeated rejection, and a group of recipients free of rejection. The chi-squared test was used as the statistical test.

Results

There were no statistical differences in age, sex, and HLA matching in the four groups of patients. The results of the study are summarized in Table 1.

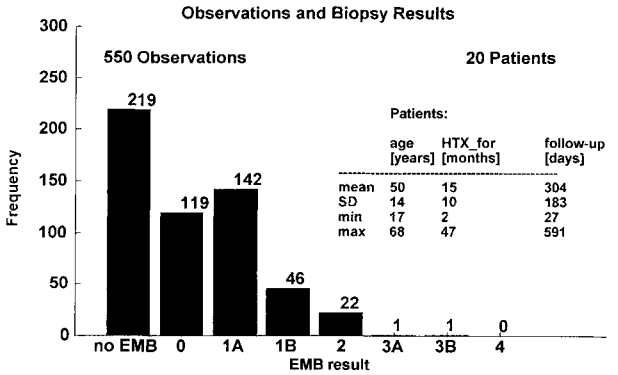

Fig. 1 Number of recordings and associated epimyocardial biopsy *(EMB)* grades, statistics. *(HTX* Heart transplantation)

Methods

Between June 1993 and February 1995, 20 consecutive patients of the heart transplant program at the University of Graz were included in the study. The mean age of patients was 50 years (SD 14 years). The mean observation time was 304 days (SD 183 days). Basic immunosuppression therapy consisted of cyclosporine (serum trough levels of 150–250 ng/ml, determined by high-pressure liquid chromatography) with additional azathioprine (1–2 mg/kg per day) and methylprednisolone (7.5 mg/day). Cytolytic therapy with anti-thymocyte globulin (ATG) was administered postoperatively for 7–14 days. Rejection episodes were treated, beginning with grade 1B [grading of The International Society for Heart Transplantation (ISHT)] [3], with pulses of methylprednisolone. Non-resolving rejections and cases with myocyte necrosis were treated with ATG or with a monoclonal interleukin-2 receptor antibody (BT 563) [8]. EMB were performed weekly during the first 3 months after heart transplantation (HTX). Depending on the patient's course, EMB intervals were extended to every 6 months thereafter. Eight pieces of myocardium were extracted with a number 9 French forceps (Scholten Surgical Instruments, Calif., USA) for each EMB and classified in accordance with the grading system of the ISHT [3].

Equipment

A commercially available VVI pacemaker (PM) with telemetric capabilities and the appropriate telemetry receiver (Mikros-Biogard and Biogard/TM2) were used with an epimyocardial screw-in electrode with fractally coated surface (ELC 54-UP). The PM system was implanted during the transplant operation, placing the electrode at the anterior wall of the right ventricle. The PM was implanted subcutaneously at the left upper abdominal wall. The telemetry system is based on analog frequency modulation and features a band-width from 0.5 to 200 Hz. The PMs were programmed with the appropriate programmer (PMS 600, all appliances mentioned so far: Biotronik, Berlin, Germany). The signals were sampled with 1200 Hz and stored using an adapted, PC-based high resolution ECG system (Predictor I, Corazonix, Okla., USA). Measured data were transferred to a UNIX workstation (DECstation 5000/240, Digital, Mass., USA) for evaluation with specially designed software for serial ECG processing.

Measurement procedure

Recordings were performed under standardized conditions (e.g. rest for 15 min, lying in supine position without moving and talking, at a time period between 2 : 00 and 3 : 00 p.m.).

A 1-min signal sequence of the telemetrically transmitted EE was recorded after the PM had been programmed to a pacing rate of 100 beats/min. Signal recording was started between the 2nd and 7th postoperative day, followed by daily recordings up to the first EMB. Thereafter, EEs were recorded on the days when EMB was scheduled. Additional recordings were taken in case of acute change of the clinical status such as acute rejection (AR) or infection episodes. Signals were recorded and processed without knowledge of the EMB result.

Data transfer

After signal recording, data were transferred to the workstation via Internet data transfer. After signal processing, data were retransferred automatically to the transplant unit within 3–5 min.

Signal processing

VER sequences were automatically processed (beat detection, beat classification, and signal averaging). Two parameters were extracted from the resulting VER sequence:

1. The VER depolarization amplitude (VERDA), defined as the magnitude of the early-negative part of the VER, typically 45 ms poststimulus.
2. The VER repolarization amplitude (VERRA); magnitude of the late-negative part of the VER, typically 350 ms poststimulus.

Statistical methods

All parameter values were expressed as percentages of an individual reference value. The reference value was computed as the mean of the parameter values of all recordings for each patient, independently of the corresponding EMB result. For statistical analysis, the normalized parameter values were grouped according to the associated EMB grades. All rejection grades ≥ 2 were subsumed in one group. For each group means and standard deviations were computed. Differences between the groups were tested via the Wilcoxon-Mann-Whitney two-tailed U-test. The threshold level of significance for the group differences was $P \leq 0.05$.

For diagnostic quality calculation, a single threshold was used as a retrospective diagnosis model. Sensitivity (SENS) and specificity (SPEC) were computed for the threshold that maximized the diagnostic quality index (DQI) for the correlation between the VER and EMB results. DQI was calculated using the following equation:

$$DQI = SENS \quad SPEC$$

A combination parameter of VERDA and VERRA, denoted VER-AREA, was calculated as the true geometric area of the signal curves in relation to the signal baseline.

Results

Except for two cases of "twiddler syndrome", no severe complications have been observed from the PM-electrode system. In these two cases, epimyocardial elec-

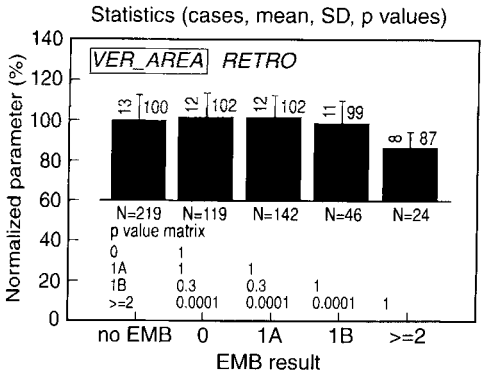

Fig. 2 Normalized parameters (%) and standard deviations of a combination parameter *(VER-AREA)* of the ventricular-evoked response depolarization and repolarization amplitudes. *P* values calculated for EMB grades 0, 1A, and 1B compared with rejection grade ≥ 2

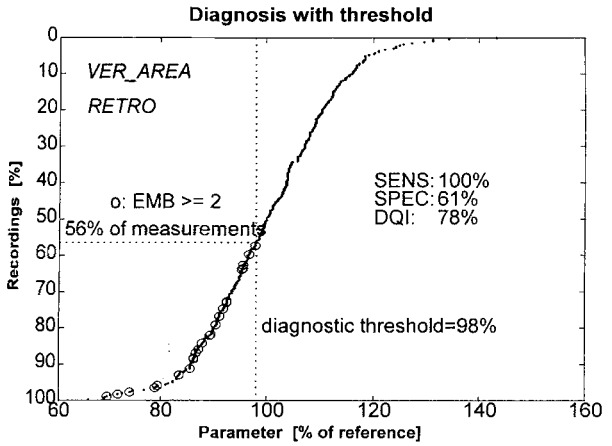

Fig. 3 Example of the application of the diagnosis model on VER-AREA with the threshold of parameter percentage that optimized the diagnostic quality index *(DQI.* The values of all recordings for all patients are plotted in ascending order (beginning in the lower left corner). *Encircled* values represent EMB grades ≥ 2. The values obtained for sensitivity *(SENS)*, specificity *(SPEC)*, and DQI are displayed

trodes have been changed to a transvenous system and the pacemaker fixed. During the investigation period, one patient died because of early graft sclerosis 126 days after HTX. A total of 550 examinations with VER recordings and 331 associated EMBs have been evaluated. Twenty-two acute rejections (ARs) with EMB grade 2, one with grade 3A and one with 3B were observed (Fig. 2).

The VER-AREA parameters did not differ comparing rejection grades 0 to 1B. The values decreased significantly due to rejection episodes of rejection grades ≥ 2 (Fig. 2). The application of the diagnosis model for VER-AREA values is shown in Fig. 3. By using a threshold as previously defined, all grade 2 rejection episodes and the two grade 3 episodes (3A and 3B) were correctly detected. A SENS of 100 %, SPEC of 61 % resulted in a DQI of 78 %. For optimizing the DQI, the diagnostic threshold was set to 98 % of the reference. In this calculation, 56 % of measurements were obove the diagnostic threshold, with no rejection greater than grade 1B.

Data transfer via Internet was performed by resident doctors trained to use PC applications. After the installation period no problems occurred with data transfer and data processing. Results, with all preceding data and the current in graph form, were available within 3–5 min.

Discussion

This study indicates a significant relationship of VER to rejection, with at least one focus of aggressive infiltration and/or focal myocyte damage as confirmed by EMB.

It has to be emphasized that most rejection episodes detected by VER analysis in this retrospective study were classified as focal moderate. The values of SENS (100 %) and SPEC (61 %) obtained must be regarded in the light of the controversially discussed reliability of EMB itself. Many studies concluded that EMB fails in some cases, at the least, SENS and SPEC do not reach 100 % simultaneously [7, 11, 18].

Since decreasing VER amplitudes were also observed in the case of infection episodes (false-positive diagnoses), additional clinical information can further increase diagnostic reliability. A higher frequency of investigations would allow a more accurate reference value to be obtained. The results of our study, however, have shown a significant accordance with EMB results with regard to the focus of interest in rejection diagnoses, the focal moderate, i.e., grade 2 rejection. Another conclusion of our results is that both pathophysiological and electrophysiological changes are related to AR, although the causal and temporal effects might be different. Longitudinal observations indicate that changes of VER parameters precede histological signs of rejection. After institution of rejection therapy, mainly with the administration of methylprednisolone pulse therapy, VER signal amplitudes increased impressively after 1–3 days; this is earlier than cellular infiltrates are expected to disappear.

Recently, it has been reported that high energy phosphate (ATP) depletion begins when focal moderate degrees of rejection occur [2]. These concordant results could give an estimate of the immunological influence on cellular metabolism and myocyte electrical activity during rejection, independent of or prior to the cellular immune system becoming affected by myocytes. Some cases have been observed in patients with compromised

circulation status, such as dilatation of the ventricles, but in the absence of histological signs of rejection or myocarditis or general infection. VER parameters dropped markedly in these cases and reelevated after reconstitution of the status due to steroid therapy. Had we been confronted with "vascular rejections" in these cases, the method presented would also have been of value.

Data transfer via Internet (during this study between two institutions in the same town) was easy to handle and gives an estimate of the practicality of a multicenter application of the method. Results of the computed data can be available within minutes from every center connected with Internet.

In conclusion, the method gives the option for a noninvasive, easily applicable and inexpensive instrument for rejection and immunosuppressive therapy monitoring which can save 50–60 % of EMBs without exposing patients to a higher risk of undetected rejection.

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