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No evidence for humoral autoimmunity against cardiomyocytes, adrenergic or muscarinic receptors in patients with Tako-Tsubo cardiomyopathy

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ABSTRACT

Background: An association between Tako-Tsubo cardiomyopathy (TTC) and underlying malignancies has been observed, suggesting that TTC might be the consequence of paraneoplastic phenomena. This study investigates the presence of autoantibodies against cardiomyocytes as well as adrenergic (β_1, β_2) and muscarinic (M2) receptors in patients with TTC.

Methods and results: Serum from 20 TTC patients and 20 controls with ischemic heart disease was obtained. Indirect immunofluorescence testing for intracellular autoantibodies against cardiomyocytes showed a homogenous distribution, as in both groups 9 of 20 sera displayed a characteristic binding pattern of antibodies including vascular walls and intracellular structures. Flow cytometry analysis revealed no difference between TTC and controls in the binding of autoantibodies to the surface antigens of cardiomyocyte HL-1 cells ($p = 0.569$, t-test). Flow cytometry analysis of nontransfected wild type cells ($p = 0.633$, t-test), M2 receptor-transfected cells ($p = 0.687$, *t*-test), β_1 receptor-transfected cells ($p = 0.444$, *t*-test) and β_2 receptor-transfected cells ($p = 0.632$, *t*-test) showed similar results for control and TTC sera. Likewise, the binding pattern of TTC patients with a history of neoplasia compared to those without or to controls did not differ significantly ($p > 0.05$, u-test).

Conclusion: Findings suggest that the presumed paraneoplastic etiology of TTC cannot be attributed to the formation of these antibodies.

1. Introduction

Tako-Tsubo cardiomyopathy (TTC) is an acquired cardiomyopathy characterized by an acute, regional but transient contractile dysfunction of the left ventricle. To date, an overarching etiological explanation for this syndrome is still missing. The most established theory includes catecholamine-mediated stunning with excessive catecholamine levels following emotional or physical stress that substantially contribute to cardiac failure (Nef, et al., 2010).

Interestingly, an association between TTC and underlying malignancies has been observed, suggesting that TTC might be the consequence of paraneoplastic phenomena (Burgdorf et al., 2008). In paraneoplastic syndromes, especially those affecting the central nervous system, cross-reactive autoimmune processes against autoantigens, shared by the tumor and healthy tissue, can be functionally active and therefore cause characteristic symptoms (Pelosof and Gerber, 2010). However, functionally active autoantibodies targeting cardiac structures, such as adrenergic receptors, have also been described in forms of myocarditis and dilative cardiomyopathy (DCM) (Caforio et al., 2008; Yoshikawa et al., 2009; Yoshizawa et al., 2012). The presence of autoantibodies against cardiomyocytes as well as adrenergic and muscarinic receptors has not yet been investigated in patients with TTC.

2. Material and methods

To address this issue, we obtained serum from 20 consecutive patients with TTC at the time point of diagnosis, diagnosed by coronary

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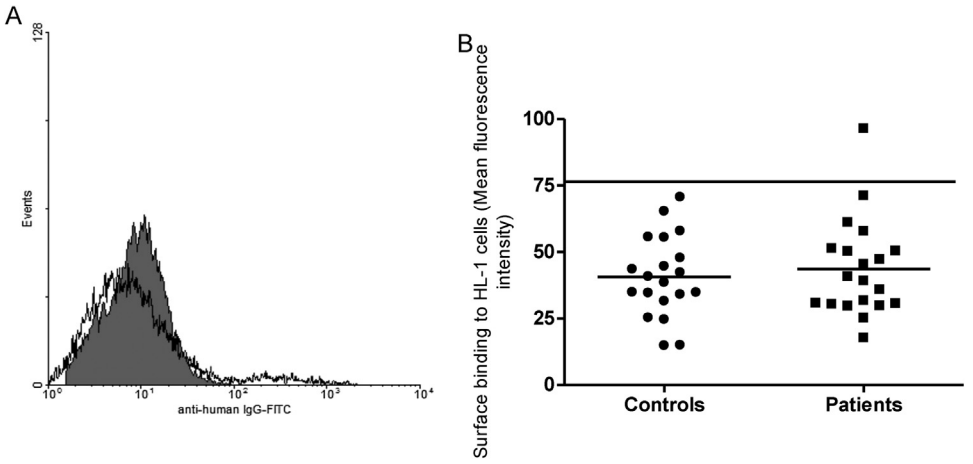

Fig. 1. (A) Binding of a TTC serum (filled graph) and a control serum (line graph) to HL-1 cardiomyocytes. (B) Binding to HL-1 cardiomyocytes (expressed as mean fluorescence intensity). Cut-off, marked as the horizontal line, was determined as the mean of controls + 2.5 SD. No difference could be seen in binding between TTC and control sera ($p = 0.569$, *t*-test).

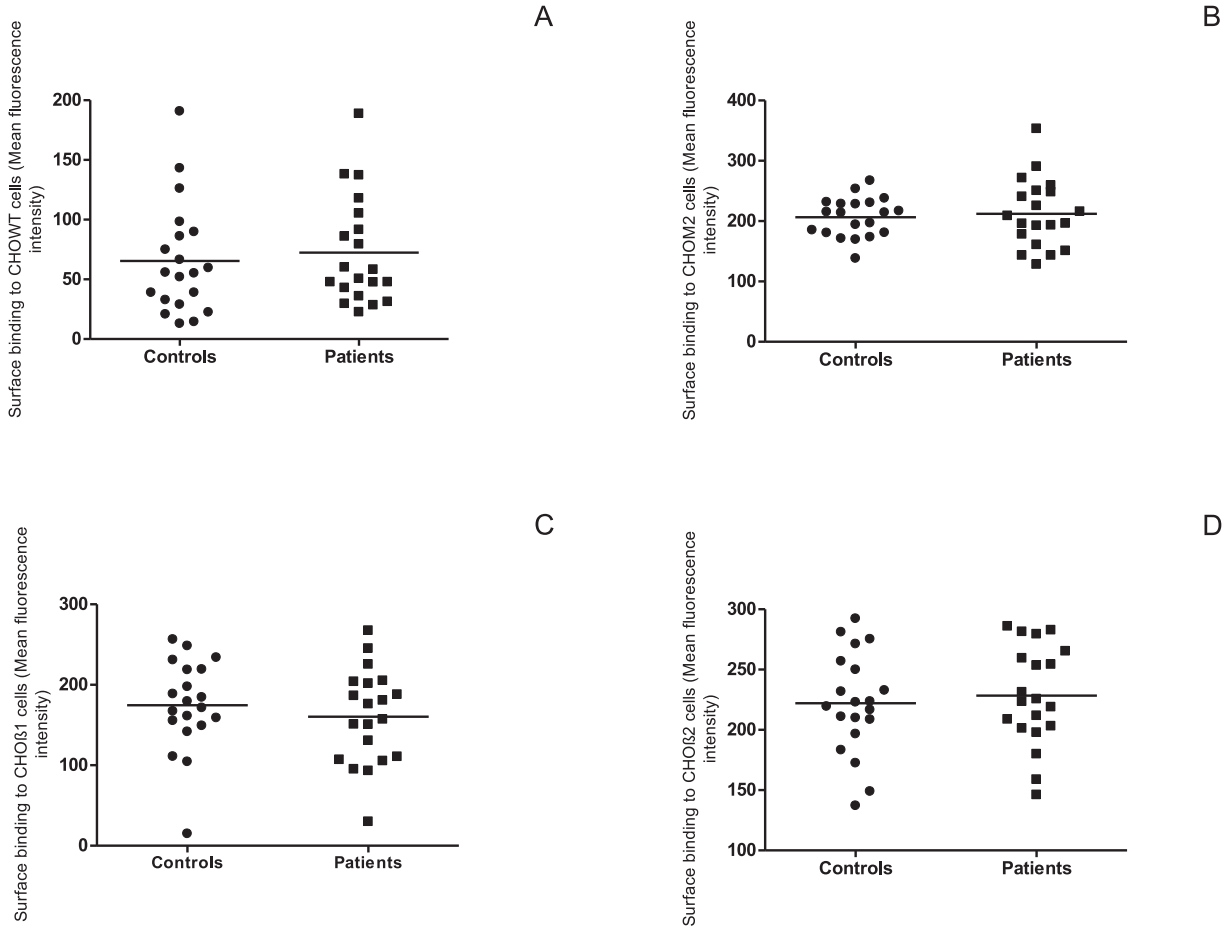

Fig. 2. Surface binding of autoantibodies to CHO cells. (A) Nontransfected WT cells ($p = 0.633$, *t*-test), (B) M2 receptor-transfected cells ($p = 0.687$, *t*-test), (C) β_1 receptor-transfected cells ($p = 0.444$, *t*-test) and (D) β_2 receptor-transfected cells ($p = 0.632$, *t*-test) were incubated with TTC and control sera; binding was analyzed by flow cytometry. No difference could be observed in the binding of TTC and control sera to different receptor types.

angiography, ventriculography, magnetic resonance imaging, and echocardiography and 20 control subjects with chronic ischemic heart disease at Kerckhoff Heart and Thorax Center, Bad Nauheim, Germany. Approval of the Institutional Review Board was obtained, and all participants gave written, informed consent.

Indirect immunofluorescence testing (IFT) for antibodies against cardiomyocytes was performed using a commercial assay with unfixed frozen sections of cardiomyocytes (Euroimmun, Lübeck, Germany). Sections were incubated with the serum sample (dilution 1/50 in phosphate buffered saline (PBS)/Tween 0.2%) for one hour at room temperature. After washing in PBS/Tween 0.2%, the sections were exposed to fluorescein isothiocyanate (FITC)-labeled goat antihuman IgG (Sigma, diluted 1/75) for 30 min, followed by two more washing steps. Immunofluorescence was viewed on an immunofluorescence microscope (Zeiss, Oberkochen, Germany). The initial serum dilution was 1/50; all samples were diluted to end-point titers in IFT. Sera positive for antinuclear, antimitochondrial and antismooth-muscle antibodies were used as positive controls.

For the visualization of cardiomyocyte surface-binding autoantibodies, fluorescence-activated cell sorting (FACS) was conducted.

The murine cardiomyocyte HL-1 cell line used for these experiments was a kind gift from Prof. William Claycomb, USA. The cells were cultured according to the provided protocol and then incubated with sera from TTC patients and controls (1:50 in FACS buffer) for 30 min at 4 °C, washed 3 times with FACS buffer (PBS, 1% fetal bovine serum (FBS) and 0.1% NaN3) and incubated again with FITC-conjugated antihuman IgG (DAKO) (30 min, 4 °C, in the dark).

To detect antibodies in TTC sera against adrenergic β_1, β_2 and muscarinic M2 receptors, we used Chinese hamster ovary (CHO) cells stably transfected with β_1, β_2 and M2 receptors (Prof. Klaus Mohr, Bonn, Germany). Nontransfected CHO cells enabled visualizing the specificity of binding to receptors. All three cell lines were cultured in Dulbecco's Modified Eagle Medium (DMEM)/Ham's F-12 containing 10% fetal bovine serum, 2 mM L-glutamine, 100 units/ml penicillin and 100 mg/ml streptomycin. The medium for stable transfected cells was additionally enriched with geneticin (63 mg/L) as the selection antibiotic. The cells were then incubated with sera from TTC patients and controls (1:50 in FACS buffer) for 30 min at 4 °C, washed 3 times with FACS buffer (PBS, 1% FBS and 0.1% NaN3) and incubated again with FITC-conjugated antihuman IgG (DAKO) (30 min, 4 °C, in the dark).

After washing again, binding of both cardiomyocyte and receptor autoantibodies was analyzed in FACScalibur (Beckton-Dickinson, Heidelberg, Germany) using CellQuest® software. Mean fluorescence intensity above +2.5 standard deviations (SDs) from the controls was considered positive for all cell lines tested.

3. Results

The study population included 19 female and one male patient in each group, with a mean age of 64 ± 11 years for TTC patients and 64 ± 6 years for controls. Drug treatment at time of inclusion included no immunmodulatory therapy. Four TTC patients had a history of neoplasias, i.e. endometrial cancer (diagnosed 2002, FIGO I, treatment: hysterectomy/adnectomy), mamma carcinoma (diagnosed 1992, treatment: surgery/chemotherapy), basalioma (diagnosed 2008, treatment: excision) and superficial spreading melanoma (diagnosed 2006, stadium Clark II, treatment: surgery). Non of the control patients had known neoplasia.

Indirect IFT for intracellular autoantibodies against cardiomyocytes showed a homogenous distribution, as in both groups 9 of 20 sera displayed a characteristic binding pattern of antibodies including vascular walls and intracellular structures. Flow cytometry analysis (Fig. 1A) revealed no difference between TTC and controls in the binding of autoantibodies to the surface antigens of cardiomyocyte HL-1 cells (40.597 ± 14.800 vs. 43.505 ± 17.832, $p = 0.569$, t-test) (Fig. 1B). Flow cytometry analysis of nontransfected wild type (WT) cells (65.375 ± 45.298 vs. 72.335 ± 43.937, $p = 0.633$, t-test) (Fig. 2A), M2 receptor-transfected cells (206.385 ± 31.409 vs. 202.196 ± 55.198, $p = 0.687$, t-test) (Fig. 2B), β_1 receptor-transfected cells (174.599 ± 55.103 vs. 161.163 ± 57.345, $p = 0.444$, t-test) (Fig. 2C) and β_2 receptor-transfected cells (222.067 ± 40.963 vs. 228.417 ± 40.238, $p = 0.632$, t-test) (Fig. 2D) showed similar results for control and TTC sera. Likewise, the binding pattern of TTC patients with a history of neoplasia compared to those without or to controls did not differ significantly ($p > 0.05$, Mann-Whitney u-test, not shown).

4. Discussion

Circulating, functionally active autoantibodies against cardiac structures (i.e., the β_1 adrenergic receptor and M2 muscarinic receptor) have been found more frequently in patients suffering from myocarditis and dilated cardiomyopathy than in controls with ischemic heart disease or healthy subjects (Caforio et al., 2008; Magnusson et al., 1994; Fu et al., 1993). In vitro studies showed that β_1 receptor antibodies can increase the contraction frequency of isolated, spontaneously beating rat myocytes, suggesting that these might contribute to the deterioration of ventricular systolic function in vivo as well (Magnusson et al., 1994). Furthermore, immunization of rabbits with synthetic peptides corresponding to the extracellular loop sequence of either M2 muscarinic or β_1 adrenergic receptors led to dilatation of ventricles within one year (Matsui et al., 1997). To date, knowledge on the presence and relevance of these autoantibodies in TTC is still lacking.

In order to close the gap between the existence of known trigger factors and the development of TTC, Burgdorf et al. conducted a case control study comparing the history of malignancies and follow-up data of 50 TTC patients and 50 control subjects with acute anterior myocardial infarction. In 16 TTC patients, including newly diagnosed neoplasias, and three patients in the control population, the authors found a significantly higher prevalence of malignancies, hypothesizing a paraneoplastic etiology for TTC, at least partly, by an increase of cardiac adrenoceptor sensitivity (Burgdorf et al., 2008). Against the background of a smaller study population in the present investigation, our results seem to support this observation, since four TTC patients (20%) and no control patient had a history of neoplasia.

Since the pathophysiology of paraneoplastic syndromes includes inter alia, pathological, functionally active antibodies caused by cross-reactivity between malignant and healthy tissue (Pelosof and Gerber, 2010), we investigated the presence of autoantibodies against cardiomyocytes as well as adrenergic and muscarinic receptors in patients with TTC compared to control subjects suffering from ischemic heart disease. Indirect immunofluorescence test for intracellular antibodies against cardiomyocytes and flow cytometry analysis for surface binding to primary cardiomyocyte HL-1 cells as well as to CHO cells expressing β_1, β_2 or M2 receptors revealed no significant differences between sera from TTC patients and controls, which seems also to apply for TTC patients with a history of neoplasia. These data suggest that the presumed paraneoplastic etiology of TTC cannot be attributed to the formation of these antibodies.

Conflicts of interest

None.

Acknowledgements

None.

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