Effects of protein A immunoadsorption on methylglyoxal levels in patients with chronic dilated cardiomyopathy and diabetes mellitus

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Abstract

Objectives: The objective of this study was to investigate effects of immunoadsorption (IA) on methylglyoxal (MG) levels in patients with chronic non-familial dilated cardiomyopathy (DCM), as well as clinical and humoral markers of heart failure.

Background: Previous studies have demonstrated favourable outcomes of IA in DCM patients with diabetes mellitus (DM) and that MG sensitizes cultured cardiomyocytes to injury by post-translational modification of Thioredoxin via glycation. Therapeutic interventions scavenging advanced glycation end-products (AGE) precursors may attenuate myocardial injury in diabetic patients. Possible effects of IA on MG levels in patients with DCM and DM have never been analyzed before.

Methods: We performed IA using agarose columns on five consecutive days in 10 patients with chronic DCM and DM, congestive heart failure of NYHA class ≥ II, left ventricular ejection fraction (LVEF) ≤ 50 %, and mean time since initial diagnosis of 4.7 ± 3.9 years.

Results: Immediately after IA, IgG decreased from 10.7 ± 1.9 to 1.1 ± 0.6 g/L (89.7 %, P = 0.008) and IgG3 from 0.6 ± 0.2 to 0.2 ± 0.2 g/L (66.7 %, P = 0.01). Median NT-pro BNP was reduced from 1665.0 ng/L at baseline to 1163.0 ng/L after 6 months (P = 0.04). Also mean LVEF was significantly improved (25.5 ± 11.7 % to 30.9 ± 11.9 % after 6 months, P = 0.02) and LVEF improved ≥ 5% (absolute) in 7 of 10 (70.0 %) patients. After 6 months, bicycle spiroergometry showed a significant increase in exercise capacity from 73.3 ± 15.8 Watts to 93.3 ± 16.4 Watts (P = 0.04) while VO2max rose from 13.0 ± 2.4 to 15.0 ± 2.2 mL/min kg (P = 0.05). No significant changes in MG levels 6 months after IA (169.6 ± 56.9 nM to 208.7 ± 75.2 nM, P = 0.05) were noted.

Conclusions: In this study on patients with nonfamilial DCM and DM, IA therapy significantly improved clinical and humoral markers of heart failure severity. However, no significant changes in MG levels were observed. Therefore, these promising results in diabetic DCM patients may not be caused by altering AGE levels. Future blinded prospective multicenter studies are necessary to identify possible underlying mechanisms like polyvalent antibodies in diabetic patients.

Key words: clinical effects, diabetes mellitus, dilated cardiomyopathy, methylglyoxal, protein A immunoadsorption

Abbreviations

advanced glycation end-products (AGE)
Angiotensin-Converting Enzyme (ACE)
angiotensin (AT)
cardiac resynchronization therapy (CRT)
C-reactive protein (CRP)
diabetes mellitus (DM)
dilated cardiomyopathy (DCM)
immunoadsorption (IA)
immunoglobulin G (IgG)
international normalized ratio (INR)
left ventricular ejection fraction (LVEF)
methylglyoxal (MG)
New York Heart Association (NYHA)
partial thromboplastin time (PTT)

Background

DM is a leading metabolic disorder associated with severe systemic consequences if poorly managed in the clinical setting. Considerable experimental and clinical studies have demonstrated the close association between diabetes and significant cardiovascular morbidity and mortality [1-4]. Methylglyoxal (MG), a highly reactive dicarbonyl, is a natural metabolite in glucose metabolism. It is capable of inducing the non-enzymatic reaction glycation, or glycosylation, between reducing sugars and proteins and other biomolecules, yielding irreversible advanced glycation end-products (AGE). Elevated MG levels are believed to contribute to complications seen in poorly controlled diabetic states [5-8]. It has been demonstrated, that MG is capable of sensitizing cultured cardiomyocytes to injury by post-translational modification of thioredoxin via glycation [9]. Additionally, recent investigations have shown that MG induces apoptosis of rat Schwann cells [10] and human vascular endothelial cells [11], indicating the significant role MG plays in the etiology of diabetic complications. Therapeutic interventions scavenging AGE precursors may attenuate myocardial injury in hyperglycemic state diseases such as diabetes. Previous studies have demonstrated favourable effects of protein A immunoadsorption (IA) in patients with chronic dilated cardiomyopathy (DCM) and DM [12,13]. This study analyzed possible effects of protein A IA on MG levels in patients with chronic DCM and DM.

Methods

Study patients

10 patients (10 male [100.0 percent of total]) with DM and chronic non-familial DCM (mean symptomatic disease duration 4.7 ± 3.9 years, no relative diagnosed with DCM, conduction defects, arrhythmias, syncope, muscle weakness, or history of first-degree relative with an unexplained sudden death under the age of 35 years [14]) were included in the study. Prior to study entry all patients had a reduced left ventricular function, i.e. LVEF below 50%, and were in New York Heart Association functional class II or worse. Detailed patient characteristics and baseline laboratory values are summarized in table 1. All patients gave written informed consent prior to study entry. Preceeding study inclusion, coronary heart disease was excluded by coronary angiography. All patients had been taking a stable dose of oral medications (beta blockers, Angiotensin-Converting Enzyme (ACE) inhibitors, angiotensin (AT) 1 antagonists, aldosterone antagonists, diuretics) for at least three months before study entry (table 1) and heart failure medication was held constant during the 6 month follow-up. Exclusion criteria were active or chronic infectious diseases, malignancies, chronic alcoholism, pregnancy, or heart failure due to other known origins.

Immunoadsorption

Immunoadsorption was performed in 10 patients with DCM, using a central venous catheter to ensure adequate flow rates, with one session daily on five consecutive days. Immunoglobulin extraction was achieved

Table 1: Patient characteristics at baseline (n=10)

parameter	mean	standard deviation
patient age [years]	58.0	4.9
time since initial diagnosis [years]	4.7	3.9
NYHA functional class	**n**	**percent of total**
NYHA II	8	80.0
NYHA III	2	20.0
cardiac resynchronization therapy device	4	40.0
parameter	**mean**	**standard deviation**
LVEF [%]	25.5	11.7
bicycle spiroergometry data: exercise capacity [Watt] VO2max [mL/min*kg]	73.3 13.0	15.8 2.4
comedication	**n**	**percent of total**
beta blocker	10	100.0
ACE inhibitor and/or AT1 antagonist	10	100.0
diuretic	8	80.0
digitalis	6	60.0
aldosterone antagonist	3	30.0
laboratory parameter	**mean**	**standard deviation**
sodium [mmol/L]	138.7	2.0
potassium [mmol/L]	4.1	0.6
calcium [mmol/L]	2.4	0.1
urea [mg/dL]	53.2	23.1
creatinine [mg/dL]	1.2	0.4
glucose [mg/dL]	131.1	24.0
haemoglobin [g/dL]	13.3	1.1
haematocrit [%]	39.1	3.0
cholesterol [mg/dL]	172.2	34.1
LDL [mg/dL]	103.3	36.3
HDL [mg/dL]	40.4	12.7
triglycerides [mg/dL]	234.4	297.0
CRP [mg/L]	7.3	5.9
total IgG	10.7	1.9
IgG 3 [g/L]	0.6	0.2
NT-pro BNP [ng/L]	1665.0 (median)	

with a protein A column (Immunosorba®, Fresenius Medical Care, Bad Homburg, Germany), as described recently [15] and ACE inhibitors were discontinued and replaced by an AT 1 antagonist 7 days prior to IA treatment. Fresenius Art-Universal was used for plasma separation and the Citem 10 monitor (Fresenius Medical Care, Bad Homburg, Ger-

many) was used to control flow through the columns. Each session was performed to yield an optimized IgG3 removal (detailed protocol, including plasma flow/volume and cycle length: table 2 [according to Fresenius Medical Care, Bad Homburg, Germany]). The 2.5-fold plasma volume was treated every day. Patients' plasma volume was calculated as previously described [16]. Patients' body weight was measured daily before and after IA treatment and diuretics were administered if patient weight following the IA session had increased by more than 1 kg. During the whole IA session full anticoagulation with unfractionated heparin was performed to avoid coagulation in the extracorporeal system (according to manufacturer's instruction, Fresenius Medical Care, Bad Homburg, Germany). Patients on warfarin included in the current study were switched to low molecular weight heparin 7 days prior to apheresis treatment. During the hospital stay full anticoagulation with unfractionated heparin was applied.

After completion of five treatment cycles (day 5), polyclonal IgG (0.5 g/kg, Octagam, Octapharma, Langenfeld, Germany) was substituted intravenously to reduce the risk of infection and to prevent rebound of antibody production. The infusion system was set at 10mL/h for 6 hours, followed by 20mL/h for the rest of the infusion until the calculated infusion volume has been reached.

Laboratory tests (including CRP, blood count, INR, PTT, and electrolytes) were performed on day 1, 3, and 5 of IA treatment to check electrolyte and coagulation status and to exclude acute infections.

Follow-up

At baseline and 6 months after IA, bicycle spiroergometry, transthoracic echocardiography, and routine laboratory tests (including electrolytes, blood count, CRP, NT-pro BNP) were obtained.

Echocardiography

Two-dimensional echocardiography was done by experienced operators, unaware of the investigation. A standardized imaging protocol was adopted with cross-sectional imaging of the left ventricle immediately distal to the mitral valve tips and apical two-dimensional imaging based on orthogonal four- and two-chamber views. 2D echo left ventricular ejection fraction was evaluated by Simpson's biplane method of discs with manual

Table 2: Immunoadsorption treatment schedule for DCM for optimized IgG 3 removal

treatment day	plasma-volume	cycle-length (min)	plasma-flow (ml/min)	duration (h:min)
1	1.5	7	25	5:25
	1	7	30	
2	1	7	30	6:18
	1.5	7	20	
3	1	7	30	6:18
	1.5	7	20	
4	1	7	30	6:18
	1.5	7	20	
5	1	7	30	6:18
	1.5	7	20	

planimetry of the endocardial border in end-diastolic (largest) and end-systolic (smallest) frames [17]. Volumes were calculated from three cardiac cycles disregarding ectopic and postectopic beats with derivation of left ventricular ejection fraction.

Bicycle spiroergometry

Bicycle spiroergometries were performed on an Oxycon Alpha system (Jaeger Toennis, Hoechberg, Germany), using a protocol starting at 0 watt with a stepwise increase of 15 watts every 2 minutes. Exercise was terminated for fatigue or other standard parameters including limiting angina pectoris, ischemic ECG changes (ST depression ≥0.3mV, ST elevation ≥0.1mV), complex arrythmias (sustained ventricular tachycardia >30s) etc. Exercise tests were obtained in the morning 2 hours after intake of morning heart failure medication.

Statistics

Data are expressed as the mean value ± standard deviation. Statistical analyses were performed with Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA). Due to limited sample size and lack of normal distribution non-parametric testing (two way: Wilcoxon Signed Rank Test, Chi-Square Test; three-way: Friedman Test) was applied for comparisons between groups. P-values of < 0.05 were considered as statistically significant.

Results

Baseline characteristics

10 patients with chronic DCM were analyzed. Mean patient age was 58.0 ± 4.9 years, and mean time post initial diagnosis was 4.7 ± 3.9 years. Median NT-pro BNP at baseline was 1665.0 ng/L. LVEF was severely reduced

(25.5 ± 11.7 percent) and exercise capacity, as assessed by bicycle spiroergometry was limited (73.3 ± 15.8 Watt, VO2max: 13.0 ± 2.4 mL/min*kg). Detailed patient characteristics including New York Heart Association (NYHA) class and baseline laboratory values are given in table 1.

Laboratory parameters

IgG reduction
Immediately after immunoadsorption IgG decreased by 89.7% (from 10.7 ± 1.9 g/L to 1.1 ± 0.6 g/L, P = 0.008) and IgG3 decreased by 66.7% (from 0.6 ± 0.2 g/L to 0.2 ± 0.2 g/L, P = 0.01).
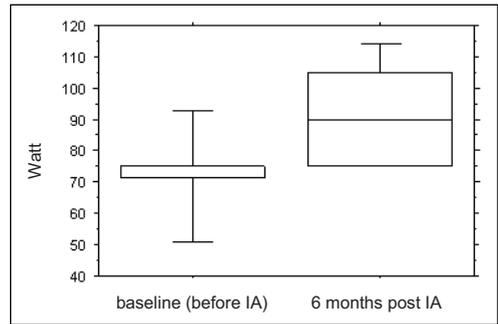
NT-pro BNP
Median NT-pro BNP decreased from 1665.0 ng/L (25th percentile 1078.3 ng/L, 75th percentile 5673.8 ng/L) at baseline to 1163.0 ng/L (25th percentile 621.0 ng/L, 75th percentile 1890.0 ng/L) after 6 months (P < 0.04). Figure 1a shows box plots of the patients' changes in NT-pro BNP concentrations. Further analysis of individual change in NT-pro BNP after 6 months revealed 5/10 patients (50.0 percent of total) with a reduction of NT-pro BNP values of ≥ 30.0 percent vs. baseline (figure 2a).

CRP
On day 5 of IA therapy, mean CRP decreased from 7.3 ± 5.9 mg/L to 3.2 ± 4.2 mg/L (P = 0.50). 9 patients (90.0 percent of total) had a reduction in CRP-levels compared to prior to apheresis therapy. No significant association between a reduction in CRP and responder status regarding LVEF or exercise capacity (VO2max, Watt) could be established (P = NS). When CRP responder status was correlated with previous (> 6.0 months before study entry) implantation of a cardiac resynchronization therapy (CRT)-device, improvement of NYHA functional class, age, time since initial diagnosis, or gender no statistically significant P-values were observed (all P = NS).
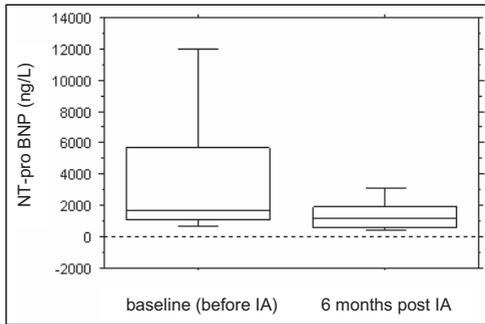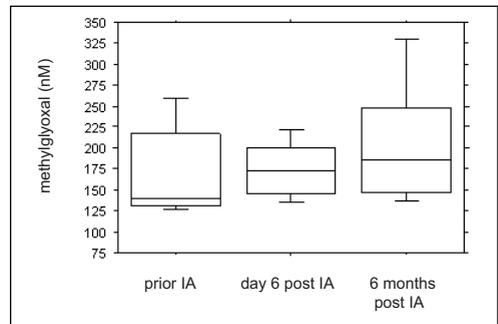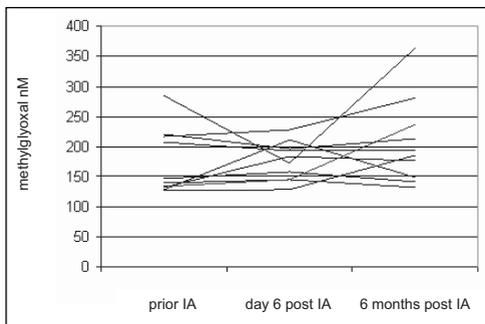
(a)

(b)
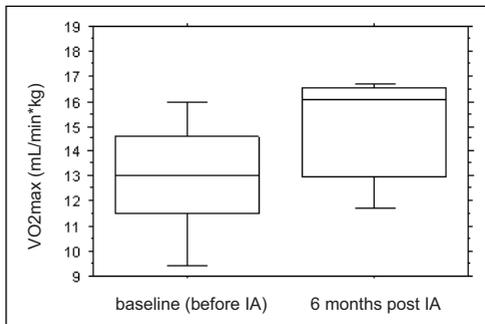
(c)

(d)

(e)

Figure 1:
a: Box plot* of NT-pro BNP (ng/L) before and 6 months after immunoadsorption therapy
b: Box plot* of exercise capacity (in Watt) before and 6 months after immunoadsorption therapy
c: Box plot* of VO2max (mL/min*kg) before and 6 months after immunoadsorption therapy
d: Box plot* of methylglyoxal (nM) before, immediately after (day 6), and 6 months after immunoadsorption therapy
e: line chart of individual methylglyoxal (nM) levels before, immediately after (day 6), and 6 months after immunoadsorption therapy
*Box plots contain the middle 50% of data, the upper edge of the box indicates the 75th percentile of the data set and the lower edge indicates the 25th percentile. Median value of the data is indicated by the line in the box and the ends of the vertical line indicate the 90th and 10th percentile.

Methylglyoxal

Mean MG levels, a precursor of AGE, were elevated at baseline (mean 169.6 ± 56.9 nM, normal range <140 nM). No significant changes were noticed in MG levels after IA (baseline 169.6 ± 56.9 nM, day 6 173.3 ± 33.2 nM, P = NS vs. baseline, 6 months post IA 208.7 ± 75.2 nM, P = 0.05 vs. baseline, Figure 1d). 40.0 percent of patients (4/10) showed a decrease in MG levels 6 months after IA, individual changes in MG are displayed in Figure 1e.

A trend towards an association between absolute decrease in MG and improvement of NT-proBNP was observed (P = 0.09). No significant association between responder status regarding MG and LVEF or exercise capacity (VO2max, Watt) could be established (P = NS). When MG responder status was correlated with previous (> 6.0 months before study entry) implantation of a cardiac resynchronization therapy CRT-device, improvement of NYHA functional class, age, time since initial diagnosis, or gender no statistically significant P-values were observed (all P = NS).

Transthoracic echocardiography

Overall, mean LV ejection fraction improved significantly from 25.5 ± 11.7 to 30.9 ± 11.9 % after 6 months (P = 0.02). Further analysis of individual change in LVEF after 6 months revealed 7/10 patients (70.0 percent of total) with an absolute improvement in LVEF of ≥ 5.0 percent vs. baseline (figure 2b).

NYHA-class

8 patients were NYHA class II (80.0 percent of total), 2 patients NYHA class III (20.0 percent of total) (table 1). 4 patients (40.0 percent of total) showed an improvement of at least one NYHA class 6 months after IA therapy.

Bicycle spiroergometry

Bicycle spiroergometry showed a statistically significant increase in exercise capacity from 73.3 ± 15.8 watts to 93.3 ± 16.4 watts (P = 0.04). Also VO2max improved from 13.0 ± 2.4 to 15.0 ± 2.2 mL/min*kg after 6 months (P = 0.05). Figures 1b-c show box plots of the patients' changes in Watt and VO2max. Anaerobic threshold was reached in all exercise tests. 60.0 percent of patients (6/10) had an increase in exercise capacity of ≥ 15 Watt (figure 2c).

Adverse events/clinical and safety laboratory parameters

Laboratory values including renal parameters did not show any statistically significant changes under immunoadsorption. During follow-up no adverse events were noticed.

Discussion

DM is closely related to cardiovascular morbidity and mortality. MG, a precursor to AGE, is increased in diabetic patient plasma and previous studies demonstrated that MG sensitized cultured cardiomyocytes to injury by post-translational modification of thioredoxin via glycation [9]. Previous own studies have demonstrated promising clinical results in diabetic patients with DCM treated with protein A IA [12,13]. We initially speculated that IA might cause a reduction in MG levels contributing to these results. Therefore we focused on MG levels after IA therapy in patients with non-familial DCM and DM. In line with previous data [13], six months after IA therapy, a significant improvement of LVEF, NT-pro BNP, and exercise capacity was demonstrated, but no significant association between reduction in MG and clinical and serological parameters of heart failure could be established.
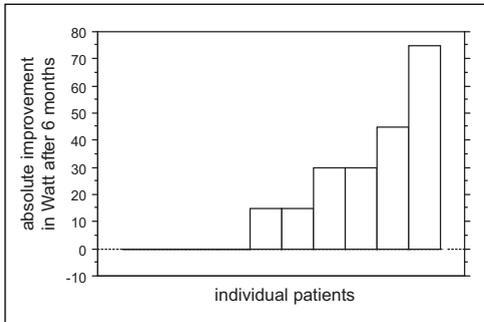
However, insulin dependent DM and DCM share common autoimmune features: a
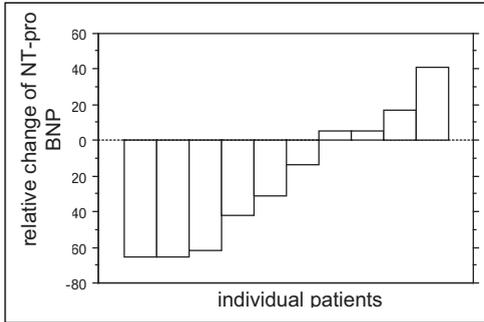
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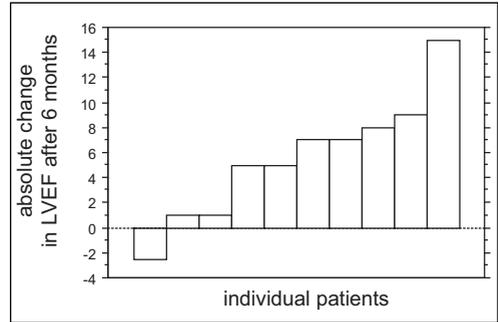
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(c)

Figure 2:
a: Relative change in NT-pro BNP (in percent) 6 months post immunoadsorption therapy
b: Absolute change in LVEF (in percent) 6 months post immunoadsorption therapy
c: Absolute change in exercise capacity (in Watt) 6 months post immunoadsorption therapy

male preponderance, a HLA-DR4 association, familial aggregation in 15-20 % of cases, and an increased frequency of the corresponding organ-specific antibodies among asymptomatic relatives [18-31]. In addition, large epidemiological studies suggest a possible relationship between DCM and DM [32,33]. These findings indicate that insulin dependent DM and DCM may have similar immunopathogenetic mechanisms, and suggest that the promising findings observed in patients with DM may be attributed to a lack of selectivity of autoantibodies against a variety of G-protein-coupled receptors [34]. Additionally, inflammatory processes appear to be involved in the pathogenesis of DCM and might represent an important factor causing progression of ventricular dysfunction. In line with previously published data [13] the vast majority of patients had lower CRP-values after IA treatment. However, most probably due to the limited number of patients no significant correlation of CRP reduction or absolute CRP values with improvement of clini-

cal or serological heart failure markers could be established.

The promising results observed might also be explained by the distinctive study population, including less severely limited DCM patients, with a shorter time since initial diagnosis compared to previous trials [12], but also by the higher amount of IgG3 reduction. As cardiodepressant antibodies belong to the IgG3 subgroup, previous studies have underlined the importance of adequate IgG3 reduction [35]. Due to application of a therapeutic protocol for optimized IgG3 reduction [15], an IgG3 reduction of 66.7 percent was obtained, which is higher compared to previous own pilot studies [12] and in line with previously described IgG3 reduction of 61 to 70 percent [13,36-38]. As recently published study data disclosed that protein A IA with ineffective IgG3 reduction shows no beneficial hemodynamic effects [37], the higher rate of IgG3 reduction might contribute to the better results of the current study [12]. However, future large blinded multicenter studies are nec-

essary to analyze effects of apheresis therapy and to rule out that similar rates of spontaneous clinical improvement might occur in a randomized control group.

Study limitations

After IA therapy, IgG was routinely substituted for safety reasons. IgG levels after substitution were comparable to the level prior to apheresis therapy. IgG treatment itself might induce beneficial effects in patients with heart failure [39], as additional effects on the immune system by IgG therapy have been observed [40]. In contrast to IgG therapy with doses of up to 2g/kg, in our study dosage of polyclonal IgG substitution was much lower (0.5 g/kg).

Various previous studies have described hemodynamic improvement of cardiac function using invasive monitoring [15,41,42]. As these studies had shown a close correlation of hemodynamic changes with non-invasive parameters of cardiac function (LVEF, seromarkers), additional application of invasive monitoring in these patients with lowered IgG plasma levels, or cardiac pool scintigraphy with application of a relevant dosage of radiation, was not performed for ethical reasons.

According to the ACC 2006 guidelines [43], some patients included in the study received a cardioverter-defibrillator prior to study entry for prevention of sudden cardiac death, prohibiting magnetic resonance imaging examinations in the present study.

Even when considering that DCM is more common in men [44], no women were present in the current study population.

Conclusions

In this study of non-familial DCM patients with DM in an earlier phase of disease, beneficial effects of IA therapy were observed, most probably due to the distinctive study population, but also due to the higher

amount of IgG3 reduction compared to previous studies [12]. MG levels were not statistically significantly affected by IA, indicating that the beneficial effects observed might not be attributed to modification of AGE-levels, but rather to polyvalent antibodies present in DM and DCM. This hypothesis is backed by recent publications regarding a lack of selectivity of autoantibodies against a variety of G-protein-coupled receptors [34], however, future blinded prospective multicenter studies appear warranted.

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