Autoantibody profiling on a plasmonic nano-gold chip for the early detection of hypertensive heart disease

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The role of autoimmunity in cardiovascular (CV) diseases has been increasingly recognized. Autoimmunity is most commonly examined by the levels of circulating autoantibodies in clinical practices. Measurement of autoantibodies remains, however, challenging because of the deficiency of reproducible, sensitive, and standardized assays. The lack of multiplexed assays also limits the potential to identify a CV-specific autoantibody profile. To overcome these challenges, we developed a nanotechnology-based plasmonic gold chip for autoantibody profiling. This approach allowed simultaneous detection of 10 CV autoantibodies targeting the structural myocardial proteins, the neurohormonal regulatory proteins, the vascular proteins, and the proteins associated with apoptosis and coagulation. Autoantibodies were measured in four groups of participants across the continuum of hypertensive heart diseases. We observed higher levels of all 10 CV autoantibodies in hypertensive subjects ($n = 77$) compared with healthy participants ($n = 30$), and the autoantibodies investigated were related to each other, forming a highly linked network. In addition, we established that autoantibodies to troponin I, annexin-A5, and beta 1-adrenegic receptor best discriminated hypertensive subjects with adverse left ventricular (LV) remodeling or dysfunction ($n = 49$) from hypertensive subjects with normal LV structure and function ($n = 28$). By further linking these three significant CV autoantibodies to the innate and growth factors, we revealed a positive but weak association between autoantibodies to troponin I and proinflammatory cytokine IL-18. Overall, we demonstrated that this platform can be used to evaluate autoantibody profiles in hypertensive subjects at risk for heart failure.

hypertensive heart disease | heart failure | autoantibodies | early detection | high-sensitivity multiplexed assay

Both the innate and adaptive immune systems play an important role in the development and progression of heart failure (HF). The association between HF and inflammation was first recognized in 1990 by Levine et al. (1) who reported elevated levels of TNF in patients with HF with a reduced ejection fraction (EF). A recent study established that patients with hypertensive heart diseases demonstrated higher levels of IL-18, a marker of inflammasome activation (2).

Whereas much research effort focused on the innate immune system, an increasing number of studies have shown that autoimmunity was also involved in HF. In the study from Latif et al. (3), IgG of antiheart antibodies was detected significantly more in patients with dilated cardiomyopathy (DCM) than in patients with ischemic heart disease. More recently, there is also evidence that autoantibodies are involved in systemic hypertension (HTN); for example, autoantibodies directed against angiotensin II type I receptors have been associated with HTN, especially in patients with pre-eclampsia (4, 5). Experimental studies support a potential role of autoantibodies in ventricular hypertrophy. Matsui et al. (6) showed that immunization of combined β1-adrenoreceptor and M2-muscarinic receptor peptides induces cardiac hypertrophy in rabbits. In another study, Wallukat et al. (7) found that aging spontaneous hypertensive rats develop antibodies against β1-adrenoreceptor. To date, however, the clinical data supporting a role for autoantibodies in hypertensive heart disease have been sparse.

Measuring cardiovascular (CV) autoantibodies remains challenging due to the lack of a standardized and sensitive assay. Moreover, because autoantibodies are often associated with different structural components or pathways, a multiplexed assay would be useful to establish the autoantibody profile. Whereas the available ELISA lacks multiplexing ability, the multiplexed Luminex platform requires relatively large amount of often precious and expensive antigens. To overcome these limitations, we have recently developed a nanotechnology-based plasmonic gold chip that affords multiplexed profiling of autoantibodies in large numbers with only ∼2-μL serum samples using down to ∼15 μL of antigens. This platform also offers high detection specificity and sensitivity, equivalent to the gold standards of RIA, owing to its ∼100-fold fluorescence enhancement ability in the near-infrared (NIR) region (8–12).

Here, we describe the development of a multiplexed specific CV autoantibody detection panel on a plasmonic gold chip for measuring key components involved in the pathophysiology of HF. Our study tests the hypothesis that CV autoantibodies are associated with hypertensive heart diseases. The discovery of new biomarkers of hypertensive heart disease is of great importance

Significance

Hypertension affects one in four adults in the United States and is a major risk factor for heart failure. A plasmonic nano-gold platform with excellent reproducibility and sensitivity is developed to address the currently unmet need of profiling circulating cardiovascular autoantibodies in hypertensive patients. We observed that patients with early-stage hypertensive heart diseases displayed a higher level of cardiovascular autoantibodies than hypertensive patients with normal cardiac function. Autoantibodies to troponin I, adrenergic beta-1 receptor, and annexin-A5 could best discriminate hypertensive heart disease. Ultimately, this nanoscience-based platform could be deployed to facilitate screening autoantibodies and identify at-risk patients for early stages of heart failure. Furthermore, this platform could have broad applications in myocarditis, heart transplantation, and other autoimmune diseases.

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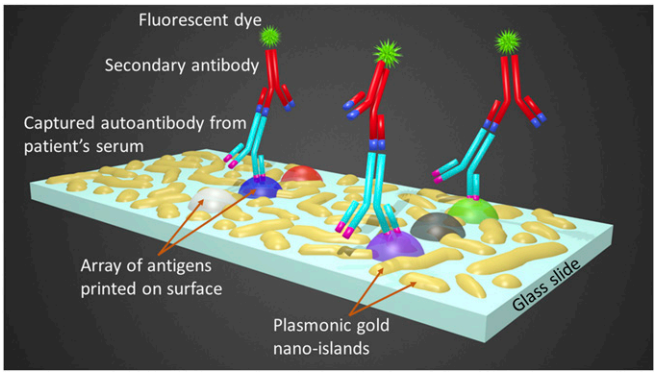

Fig. 1. The multiplexed detection of CV-specific IgG autoantibodies on plasmonic gold chips using an ultralow volume of serum samples. Schematic depicting the plasmonic nano-gold islands deposited on glass slide surface, the sandwich microarray structure, and the actual assay steps including (*i*) the immobilization of an array of CV antigens on the chip, (*ii*) the capture of the targeted autoantibodies from diluted patients' serum, and (*iii*) the binding of secondary detection antibodies conjugated with IRDye 800 fluorescent dye for signal reporting.

because routine markers such as B-type natriuretic peptide lack sensitivity for the detection of early-stage hypertensive heart diseases (13). We compared the prevalence of CV autoantibodies in four groups of subjects using a nested case control study design building on a large, well-phenotyped population-based study (14, 15). The selected groups of subjects represented a continuum of hypertensive heart diseases including healthy participants, participants with HTN but without left ventricular (LV) hypertrophy or diastolic dysfunction, and participants with systemic HTN and evidence of LV involvement with or without clinical HF or ischemic events (CV events).

Results

Development of CV Autoantibody Detection Panel on Plasmonic Gold Chips. We recently developed a plasmonic gold chip composed of a layer of nanostructured gold islands deposited on a glass surface through a solution phase growth method. The gold nanoislands were separated by nano-gaps (~10 nm), affording strong local electric-field and surface plasmon resonance enhancements to fluorescent signals in the NIR region of 650–800 nm by up to 100-fold (8–12).

Here, we constructed a sandwich immunoassay on this platform for specific CV autoantibody quantification in a microarray format by first immobilizing on the plasmonic gold chip 10 CV antigen targets that were associated with different pathways related to HF (Fig. 1). The antigens were categorized into four groups: (*i*) the structural myocardial cellular proteins [i.e., troponin I, titin, β-chain myosin heavy chain 7 (MYH7), and α-chain myosin heavy chain 6 (MYH6)]; (*ii*) the neurohormonal regulatory proteins [i.e., beta 1-adrenegic receptor (ADRBK1), angiotensin II receptor 1 (AGTR1), and cholinergic receptor muscarinic 2 (CHRM2)]; (*iii*) the vascular proteins endothelin receptor A and B (EDNR-A and -B); and (*iv*) coagulation and apoptosis protein annexin-A5. An ultralow volume of 2 μL of each human serum was diluted and incubated over the plasmonic gold chip with printed antigens, during which the corresponding human IgG autoantibodies from a patient's serum sample were captured on the target antigen spots. The abundance of each autoantibody in serum was quantified by measuring the signal of IRDye 800 fluorescence on the spots after incubating the spots with antihuman IgG labeled with IRDye 800 fluorophores (Fig. 1 and *SI Construction of CV Autoantibody Detection on Plasmonic Gold Platform*).

Detection of Antibodies on Plasmonic Gold Chips. To establish the dynamic range and sensitivity of antibody detection on plasmonic gold chips we generated calibration curves of antibodies to troponin I and CHRM2. By using commercially available samples with known concentrations of antibodies to troponin I and CHRM2 we obtained and repeated the calibration of both antibodies on three different plasmonic chips and on glass slides. We observed ~100-fold NIR fluorescence enhancement on plasmonic gold chips compared with glass, accompanied by a three-orders-of-magnitude increase in detection dynamic range and sensitivity with signal linearity down to ~10 fg/mL (Fig. 2 *A* and *B*). The coefficient variation of calibration curves obtained from different plasmonic chips in independent experiments was less than 14%. Each of the two commercial troponin I and CHRM2 antibodies was also tested individually over the 10 printed target antigens for specificity checking (Fig. 2 *C* and *D*). For the microarray probed with troponin I antibody, positive fluorescence signals were only detected on the troponin I antigen spots and there was no signal on any other antigen spots in the microarray even at high concentrations of troponin I antibody (Fig. 2*D*). We observed the same trend in the microarray probed only with CHRM2 antibody (Fig. 2*D*).

For signal normalization purposes and assay quality control the serum sample of a patient with sustained HTN and LV remodeling was included on every plasmonic gold chip used. Thus, we obtained the autoantibodies signals from the same sample 30 times on different chips from independent experiments. The calculated coefficient of variations for the detection of each of the 10 specific autoantibodies in this control sample were 8–13% (Table S1).

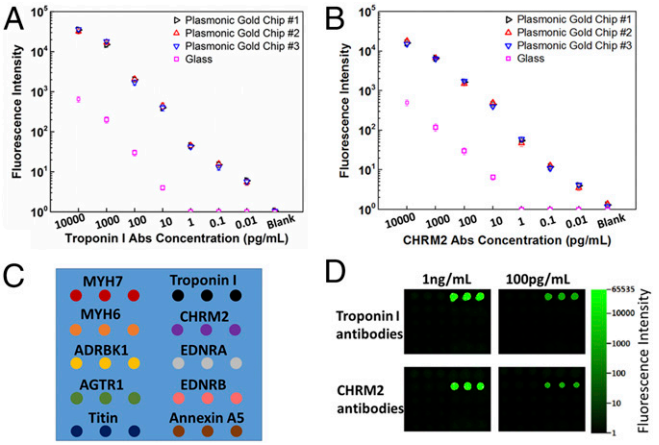

Fig. 2. The dynamic range, sensitivity, and specificity of multiplexed antibody detection on plasmonic gold chips. Calibration curves for antibody quantification and for comparing dynamic range and sensitivity of the detection of (*A*) antibodies to troponin I and (*B*) antibodies to CHRM2 on plasmonic gold chips and on glass. Samples used for generating the calibration curves were commercial standards with known concentrations of antibodies. Each experiment was performed three times, and the error bars represent the SD. The calibration curve of each antibody was repeated on plasmonic gold chips produced from various batches and the coefficient variation was <14%. (*C*) Schematic of the 10-plexed antibody detection microarray containing the 10 target antigens immobilized on a gold plasmonic chip for interacting with each of the two commercial troponin I and CHRM2 antibodies. (*D*) Fluorescence images of the two microarrays on a plasmonic gold chip probed with 1 ng/mL and 100 pg/mL troponin I and CHRM2 antibodies separately. Each array was subsequently detected with 2 nM IRDye 800-labeled secondary antibodies. Fluorescent signals were only observed on the troponin I antigen spots in the microarray probed with troponin I antibodies (*Top*), whereas only CHRM2 spots showed signals in the microarray probed with CHRM2 antibodies (*Bottom*).

Clinical Cohort. From a well-characterized population-based study with available baseline echocardiography and circulating CV biomarker measurements of subjects (Tables S2 and S3) we identified 49 patients with sustained HTN and LV concentric remodeling, hypertrophy, and/or diastolic dysfunction (LV+), of which 20 patients had a prior history of a CV event (LV+/CV+) (14, 15). In the LV+/CV+ group, 9 out of 20 patients reported a previous history of cardiac diseases before the baseline examination. During the follow-up period, nonfatal CV events occurred in 18 patients from this group. Overall, five patients experienced a coronary (myocardial infarction and/or PTCA) event, six patients had symptomatic HF, and seven patients experienced vascular (stroke or peripheral arteries revascularization) events. Then, we selected 29 age-matched (within 10 y) patients with sustained HTN but without LV involvement (LV−). In addition, we selected 30 age-matched (within 10 y to HTN LV+ patients) healthy, normotensive subjects (NTN).

CV Autoantibody Distribution in HTN Cases and Controls and Network Analysis. Patients with HTN and LV remodeling or dysfunction (LV+/CV− and LV+/CV+) displayed higher levels of high-sensitive cardiac troponin T and annexin-A5 compared with NTN controls and HTN patients without LV involvement (LV−). In addition, insulin levels were also higher in patients with hypertensive heart disease (Table S2).

Fig. 3 shows the geometric means and distributions of the mean fluorescence intensity (MFI) signals of the 10 autoantibodies measured in serum samples of three groups of HTN subjects and one group of healthy participants. We identified that all autoantibodies measured could distinguish the healthy group from the HTN subjects. Autoantibodies to troponin I, annexin-A5, and

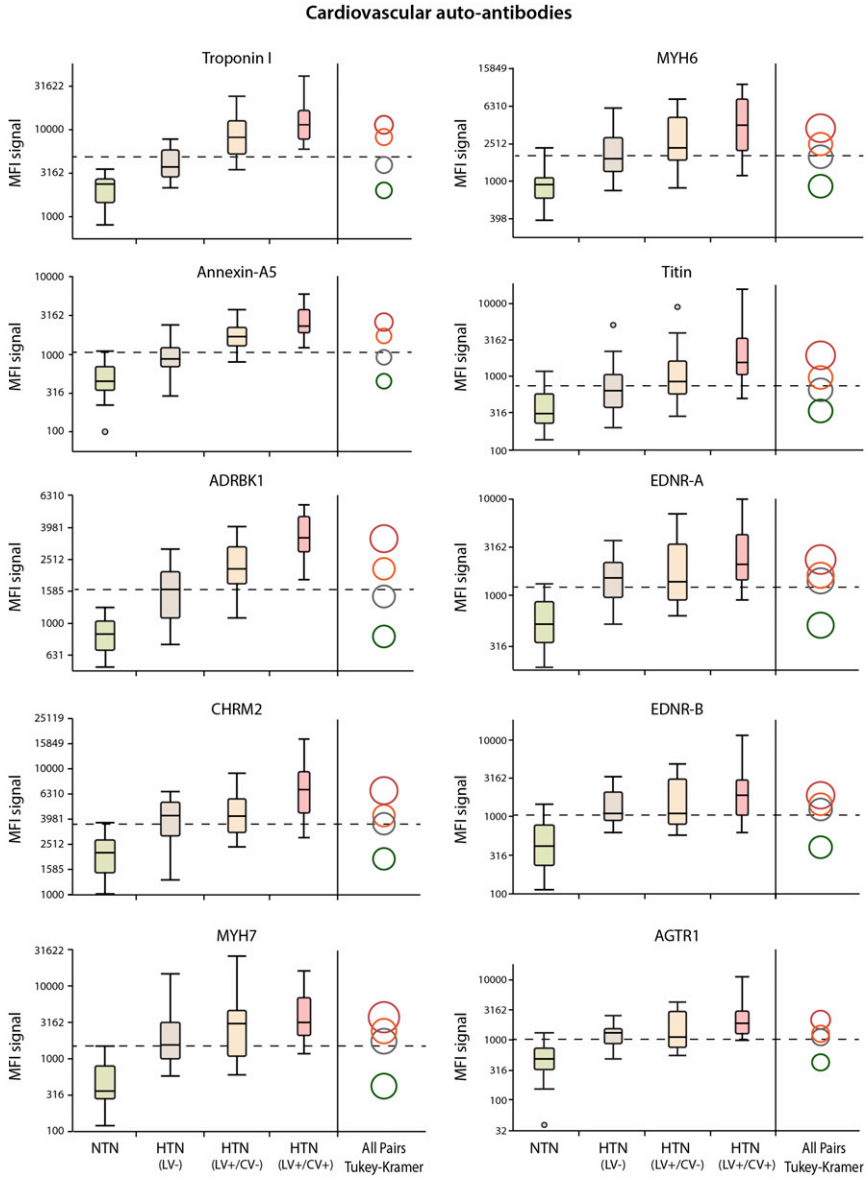

Fig. 3. Box plots of distributions of the MFI signals of the 10 measured autoantibodies in three groups of hypertensive subjects and healthy participants. Each pair of group means could be compared visually by examining the intersection of the comparison circles. Circles for means that are significantly different between groups either do not intersect or intersect slightly. If the circles intersect by an angle of more than 90°, the means are not significantly different between groups. The radii of the circles depend on the biomarker distribution, the number of tests, and number of subjects per group. The autoantibodies to troponin I, annexin-A5, and ADRBK1 were the most significant markers that best discriminated these four groups of subjects.

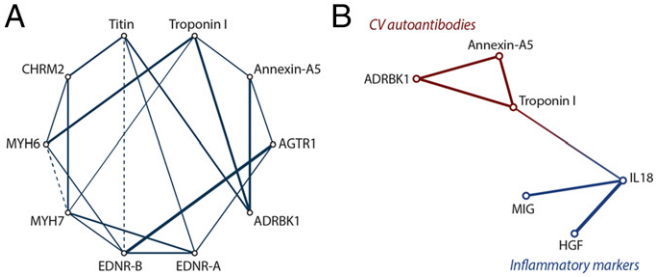

Fig. 4. Partial correlation diagrams illustrating (*A*) the intracorrelations among the 10 measured autoantibodies and (*B*) the correlation between selected CV autoantibodies and proinflammatory cytokines in all participants. Solid lines represent significant ($P < 0.01$ for all) direct correlations, and dashed lines show significant inverse correlation. Thicker lines correspond to stronger relationships. HGF, hepatocyte growth factor; MIG, monokine induced by gamma IFN.

ADRBK1 were the most significant markers that best discriminated these four groups of subjects.

We also observed significant intracorrelations among the 10 measured autoantibodies (Fig. 4*A*). We explored this by constructing partial correlation diagram in which partial correlations were estimated and each variable was plotted as a node (*SI Statistical Methods*). The strongest correlation ($r = 0.54$; $P < 0.0001$) was observed between autoantibodies to AGTR1 and EDNR-B. Autoantibodies to ADRBK1 were significantly and directly associated with autoantibodies to troponin I, annexin-A5, and titin ($r \geq 0.33$; $P < 0.0001$). Autoantibodies to troponin I also displayed strong and direct correlation to autoantibodies to MYH6 ($r = 0.40$; $P < 0.0001$).

Discrimination Analyses. We applied the partial least squares-discriminant analysis (PLS-DA) model for identifying CV autoantibodies responsible for group separation (16). First, we compared HTN patients with adverse LV remodeling or dysfunction (LV+) vs. HTN patients without LV involvement (LV−). Then, we compared healthy controls vs. HTN patients with LV involvement. For each group comparison we identified the minimum number of uncorrelated latent factors derived from the measured autoantibodies, which explained from 57.4 to 89.3% of the variations between groups ($P \leq 0.006$ for all; Table 1).

The accuracy of the discrimination model was assessed using the number of correctly classified samples and the receiver operating characteristic (ROC) plots (*SI Statistical Methods*). We established that the area under the ROC curve (AUC) for the discrimination between HTN (LV−) and the two groups of HTN (LV+) patients with or without CV events was 0.76 and 0.88, respectively ($P \leq 0.006$) and the AUC for the discrimination between healthy controls and the two groups of HTN (LV+) patients was as high as 0.96 ($P < 0.0001$) (Table 1).

Next, the importance of each autoantibody in the construction of the latent variables was assessed using the variance importance in projection (VIP) scores (*SI Statistical Methods*) and we identified the most significant autoantibodies responsible for group discrimination. Autoantibodies to cardiac-specific troponin I and annexin-A5 showed higher MFI signal in HTN patients with LV remodeling or dysfunction (LV+) compared with the HTN patients without LV involvement (LV−) (Table 1). For comparisons between HTN (LV+) patients with CV events (LV+/CV+) and HTN (LV−) patients, in addition to autoantibodies to troponin I and annexin-A5 we identified autoantibodies to ADRBK1 as another important biomarker for group discrimination (Table 1). These autoantibodies remained significant when we compared HTN (LV+) patients and healthy subjects.

With adjustment for sex, age, and body mass index (Table S2), level of CV autoantibodies to troponin I ($P = 0.0009$), annexin-A5 ($P = 0.0004$), and ADRBK1 ($P = 0.002$) remained considerably different between HTN (LV+) patients and HTN (LV−) patients. With additional adjustments of the model for the serum level of two cardiac-specific biomarkers, NT-proBNP and troponin T (Table S2), the significance of the above-mentioned CV autoantibodies still persisted.

In analyzing the relationship between autoantibodies and innate system activation we observed that autoantibodies to troponin I, annexin-A5, and ADRBK1 were linked to the innate and growth factors determined by other platforms (17). In particular, there was a significant but weak positive correlation between autoantibodies to troponin I and proinflammatory cytokine IL-18 ($r = 0.09$; $P = 0.012$) (Fig. 4*B*).

Temporal Stability of the Measured Autoantibodies. Two echocardiographic examinations were available for each of the 65 participants from the LV diastolic dysfunction group, the normal diastolic function group, and the NTN group. We tested the temporal stability of all 10 CV autoantibodies in baseline and follow-up serum samples in these 65 subjects. The median time interval between two blood samplings was 4.7 y (5th to 95th percentiles are 3.7–5.4 y). The analysis of the repeated biomarkers measurements from two visits showed that the intraclass correlation coefficient (ICC) was over 93.7% for the measured

Table 1. Summary data for each of the models generated by PLS-DA

Characteristics	Group comparison			
	HTN (LV+/CV-) vs. HTN (LV−)	HTN (LV+/CV+) vs. HTN (LV−)	HTN (LV+/CV−) vs. NTN	HTN (LV+/CV+) vs. NTN
No. of used latent factors	3	2	3	3
% of variation explained by latent factors				
For predictor variables (CV autoantibodies)	69.0	72.4	85.8	90.1
For outcome variables (LV remodeling)	57.4	68.0	82.0	89.3
AUC	0.88	0.76	0.94	0.96
No. of correctly classified	0.83	0.71	0.86	0.90
P_{AUC} value	<0.0001	0.0062	<0.0001	<0.0001
Top CV autoantibodies responsible for class discrimination	Troponin I (VIP = 1.50; CC = 0.59) Annexin-A5 (VIP = 1.38; CC = 0.49)	Troponin I (VIP = 1.34; CC = 0.26) Annexin-A5 (VIP = 1.25; CC = 0.22) ADRBK1 (VIP = 1.25; CC = 0.23)	Troponin I (VIP = 1.11; CC = 0.31) Annexin-A5 (VIP = 1.13; CC = 0.39) ADRBK1 (VIP = 1.18; CC = 0.40)	Troponin I (VIP = 1.08; CC = 0.27) ADRBK1 (VIP = 1.18; CC = 0.48)

CC, correlation coefficient for centered and scaled data.

autoantibodies ($P < 0.0001$ for all; Table S4) (18). This implies that the selected autoantibodies in this study were relatively stable between the two time points in comparison to the variability observed across the different individuals in the study.

CV Autoantibody Distribution in Subjects with DCM and Healthy Controls. We measured CV autoantibodies in 23 subjects with DCM as well as 30 healthy control subjects (*SI CV Autoantibody Distribution in Subjects with DCM and Healthy Controls*). The distribution of the MFI signals of the 10 measured autoantibodies in the sera of DCM subjects and in the healthy subjects is presented in Fig. S1. We observed that all autoantibodies measured were able to discriminate the two groups of subjects.

Discussion

We developed a high-sensitivity platform for the detection of CV autoantibodies; this multiplexed assay targeted key structural cardiac proteins as well as several key pathways involved in the pathophysiology of HF. We demonstrated that CV autoantibodies could discriminate patients with hypertensive heart disease and that autoantibodies to troponin I, ADRBK1, and annexin-A5 were the most discriminatory. In addition, we have shown that our CV autoantibody detection platform can be used to detect autoantibodies in patients with DCM.

The plasmonic gold chips had high reproducibility and consistency for measurements of CV autoantibodies. The coefficient of variation for the calibration curves of antibody detection in independent experiments was less than 14% even at a low concentration of antibodies (Fig. 2). Although ELISA is the standard for protein quantification and has been widely used for research and clinical practice, it requires a relatively large amount (submilliliters) of serum from an individual subject. In addition, both ELISA and Luminex assays require hundreds of microliters to milliliters of CV antigen (50 μL for up to $1,000) for single-autoantibody detection, making these techniques much more expensive for the detection of large numbers of autoantibodies. In contrast, the plasmonic gold chip offers the advantage of multiplexed profiling and quantification of autoantibodies using a very low serum sample volume of ∼2 μL for hundreds to thousands of samples with down to ∼15 μL of antigens.

The three autoantibodies that best differentiated hypertensive heart diseases emerged from our study were autoantibodies to troponin I, annexin-A5, and ADRBK1. Autoantibodies to titin also provided some discrimination for hypertensive heart diseases. Autoantibodies specific to the vasculature such as autoantibodies to EDNR-A and EDNR-B as well as CHRM2 or AGTR1 were more closely associated with hypertensive status than with hypertensive heart diseases (Fig. 3 and Table 1). Our study established that the discriminatory ability of CV autoantibody profiling was highly promising and autoantibody profiling could complement B-type natriuretic peptide or high sensitivity troponin for the diagnosis of early stages of hypertensive heart disease (13).

Because cardiac troponins are the biomarkers of choice for the assessment of myocardial injury, several previous studies analyzed the autoantibodies against cardiac-specific troponin I and T using the classical ELISA method or affinity chromatography with surface plasmon resonance analysis (19). The reported prevalence of autoantibodies to cardiac-specific troponin I varies from 7.3% in patients with acute coronary syndrome up to 27.9% in patients with cardiomyopathies (20–22). Of note, the experimental study showed that autoimmune response to recombinant murine cardiac troponin I induced inflammation in the myocardium followed by fibrosis and HF in mice (23). Therefore, cardiac-specific autoantibodies against troponin I might also be considered as a mediator of cardiotoxicity, with further validation needed in hypertensive heart diseases.

Circulating annexin-A5 as well as antibodies directed against annexin-A5 were associated with evidence of hypertensive heart diseases. Annexin-A5 is a cellular protein in the annexin group that is commonly used as a biomarker to detect apoptotic cells and may also play a role in the inhibition of blood coagulation. Using annexin-A5 knockout mice, Schurgers et al. (24) demonstrated that annexin-A5 is highly expressed in organs that are often affected by HF including lung, kidney, liver, and spleen. The authors suggested that cell lysis in these organs due to hypoperfusion resulted in a marked and significant increase in annexin-A5 concentrations. Along similar lines, in our study we observed that a higher level of circulating annexin-A5 in hypertensive patients with LV remodeling was accompanied by an increased level of annexin-A5 autoantibodies (Fig. 3, Table 1, and Table S2). Interestingly, antibodies against annexin-A5 were found in patients with the antiphospholipid syndrome (25). In this disease, antibodies to annexin-A5 can cause enhanced coagulation and thrombosis might disrupt the formation of the shield around negatively charged phospholipid molecules at the cell surface.

The beta-adrenergic system is a key regulatory component in HF, and several studies have shown that the adrenergic system is highly activated with advancing stages of HF (26). The fact that the production of beta-adrenergic autoantibodies increased with the development of HTN with LV hypertrophy or diastolic dysfunction (Fig. 3 and Table 1) suggested a potential role in its pathophysiology. Our results are consistent with the prior experimental studies from Matsui et al. (6) and Wallukat et al. (7), suggesting a role of anti-β1 adrenergic antibodies in the development of ventricular hypertrophy. Novel therapies targeting the anti–beta-1-adrenergic receptor antibodies could also offer an opportunity for future research and development in this area (27).

Our study profiles autoantibodies against titin, a key structural myocardial protein (28). Mutations to titin are recognized as an important cause of DCM (29). We observed that autoantibodies to titin differed between the groups of HTN patients with or without LV hypertrophy or diastolic dysfunction (Fig. 3 and Table 1), but they were not the most discriminatory autoantibodies overall. More studies are needed to test different titin antigens because this can influence the sensitivity of the assay.

The different CV autoantibodies in our study were not independent of each other but were linked through a strong network (Fig. 4*A*). In particular, autoantibodies to troponin I were significantly and directly related to MYH6 and ADRBK1 autoantibodies, whereas EDNR-B autoantibodies displayed the strongest direct correlation with AGTR1 autoantibodies. In a previous study we have shown that IL-18, a cytokine reflecting inflammasome activation, as well as HGF and CXCL9, was associated with cardiac remodeling and dysfunction in patients with HTN (17). Our findings suggested that there could be a link between autoimmunity and inflammasome activation in cardiac maladaptation in early stages of hypertensive heart diseases. This model is an emerging concept in several immune-mediated diseases (30). Moreover, several studies suggest that inflammasome activation can lead to the production of CV autoantibodies that can further cause the progression of disease via proinflammatory, prothrombotic, and cytotoxic effects (31, 32).

The analysis of the repeated biomarkers measurements from two visits showed that the ICC was high for all measured autoantibodies (Table S4). It should be noted, however, that each pair of the baseline and follow-up samples were tested side-by-side on the same chip simultaneously; therefore, the interchip variation was not reflected in this analysis of temporal stability. Over the follow-up period of 4.7 y, we confirmed that all these participants remained in the same category and no target organ damages or worsening of patients' conditions were observed.

Our study also tested the presence of autoantibodies in the other spectrum of HF, that is, DCM (Fig. S1). Previous studies suggested a role for autoimmunity in the pathogenesis of DCM (30). Latif et al. (3) measured heart-specific autoantibodies

including myofibrillar proteins by gel electrophoresis (SDS/PAGE) and Western blotting in patients with DCM or with ischemic heart disease. The study concluded that patients with DCM had a significantly greater frequency and reactivity of IgG autoantibodies against six myocardial proteins than patients with ischemic heart disease. In our study, the nanotechnology-based plasmonic gold platform was very sensitive in detecting circulating autoantibodies in patients with both ischemic and nonischemic cardiomyopathy and could potentiality be used to monitor immunomodulatory therapy and for risk stratification purposes in the future.

In conclusion, we developed a multiplexed specific CV autoantibody detection platform on a nanotechnology-based plasmonic gold chip and successfully applied it in the evaluation of CV autoantibody profiles in HTN patients with cardiac maladaptation for potential diagnostic and prognostic purposes. Autoantibodies to troponin I, annexin-A5, and ADRBK1 emerged as the most strongly associated with LV remodeling and dysfunction in patients with HTN. The panel we have developed could also be promising for application in different fields of cardiology, including myocarditis, dilated cardiomyopathies, pulmonary arterial HTN, and heart transplantation after further optimization and validation.

Materials and Methods

SI Materials and Methods details the materials and methods followed in this paper. *Supporting Information* contains protocol for the construction of the CV autoantibody detection panel on a plasmonic gold platform, an explanation of the case control study, the statistical methods used for data analysis, a description of the echocardiography, and the test results on DCM patients. Each patient gave informed consent for all experiments. The experiments were approved by Leuven University and Stanford University Institutional Review Boards.

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