TITLE PAGE

Title:

Endothelial dysfunction and glycocalyx shedding in heart failure: insights from patients receiving cardiac resynchronisation therapy.

Brief Title: Glycocalyx shedding in systolic heart failure

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Abstract

Objectives:

To determine (a) whether chronic heart failure with reduced ejection fraction (HFrEF) is associated with increased glycocalyx shedding; (b) whether glycocalyx shedding in HFrEF with left ventricular dyssynchrony is related to inflammation, endothelial dysfunction and/or redox stress and is ameliorated by cardiac resynchronisation therapy.

Background:

Glycocalyx shedding has been reported to be increased in heart failure and is a marker of increased mortality. Its role in dyssynchronous systolic heart failure and the effects of cardiac resynchronisation therapy (CRT) are largely unknown.

Methods :

Twenty-six patients with dyssynchronous HFrEF were evaluated before and 6 months after CRT insertion. Echocardiographic septal to posterior wall delay (SPWD) assessed intra-ventricular mechanical dyssynchrony, and quality of life, integrity of nitric oxide (NO) signalling, inflammatory and redox-related biomarkers were measured. Glycocalyx shedding was quantitated via plasma levels of the glycocalyx component, syndecan-1.

Results:

Syndecan-1 levels pre-CRT were inversely correlated with LVEF ($r=-0.45$, $p=0.02$) and directly with SPWD ($r=0.44$, $p=0.02$), QOL ($r=0.39$, $p=0.04$), plasma NT-proBNP ($r=0.43$, $p=0.02$), and the inflammatory marker, symmetric dimethylarginine (SDMA) ($r=0.54$, $p=0.003$). On multivariate analysis, syndecan-1 levels were predicted by SPWD and SDMA ($\beta=0.42$, $p=0.009$ and $\beta=0.54$, $p=0.001$, respectively). No significant correlation was found

between syndecan-1 levels and other markers of endothelial dysfunction/inflammatory activation. Following CRT there was no significant change in syndecan-1 levels.

Conclusions:

In patients with dyssynchronous HFrEF, markers of glycocalyx shedding are associated with magnitude of mechanical dyssynchrony and elevation of SDMA levels and inversely with LVEF. However, CRT does not reverse this process.

Keywords: Glycocalyx shedding, cardiac failure, resynchronization therapy, endothelial function, symmetric dimethylarginine

List of abbreviations:

CRT: cardiac resynchronisation therapy

SPWD: Septal to posterior wall delay.

QOL : Quality of life

LVEF: Left ventricular ejection fraction

NT-proBNP: N-terminal pro brain natriuretic peptide

AI_X : Augmentation index

NO: nitric oxide

SDMA: Symmetric dimethylarginine

TXNIP: thioredoxin interacting protein.

Introduction

In spite of several ground-breaking advances in the understanding and management of heart failure over the last few decades, HFrEF remains common in most Western societies with substantial associated morbidity, mortality and economic burden [1]. This underscores the need for further understanding of the pathophysiological mechanisms of heart failure. It is known that chronic heart failure is associated with immune and inflammatory activation [2], as well as with vascular endothelial dysfunction [3] with its associated potential for disturbance in laminar blood flow [4]. There is increasing evidence that shedding of the vascular endothelial glycocalyx may represent a link between these two processes [5], largely via activation of matrix metalloproteinases [6] and resultant induction of endothelial dysfunction. However, the extent of endothelial glycocalyx shedding in patients with severe HFrEF and concomitant left ventricular dyssynchrony, and its putative association with nitric oxide signalling and inflammatory activation, remain largely unexplored. Importantly, a direct correlation between glycocalyx shedding and loss of laminar blood flow has been observed [7].

Over the last two decades, cardiac resynchronisation therapy (CRT) has emerged as the standard of care in patients with HFrEF associated with concomitant dyssynchronous ventricular contractions, especially with left bundle branch block. CRT offers symptomatic benefit to approximately two-thirds of recipients; several landmark trials have shown that CRT, with or without defibrillator insertion, not only improves ‘soft’ clinical end-points, but also reduces risk of mortality and reduces heart failure hospitalisations [8, 9]. The basis for this benefit of CRT remains uncertain but it is thought that reduction in dyssynchrony contributes to improvement in clinical status [10]. Nonetheless, large multi-centre prospective trials have suggested that no echocardiographic measure of dyssynchrony is sufficient by itself to predict response to CRT [11].

Recently, efforts to understand the mechanisms of benefit associated with CRT have begun to address factors beyond improvement in left ventricular contractile efficiency, turning to possible effects on peripheral vascular function [12-14]. It is now known that the integrity of the endothelial glycocalyx layer plays a prominent role in maintenance of vascular endothelial function by modulating vascular permeability, coagulation, vascular tone, and complement activation [15]. Increased glycocalyx shedding occurs in conditions of increased shear and oxidative stress [7]. Release into plasma of syndecan-1, a marker of glycocalyx shedding and thus of endothelial dysfunction, has been found to be increased in patients with heart failure [16]. This elevation of plasma syndecan-1 concentrations is also a correlate of increases in the combined end point of all-cause mortality and hospitalised heart failure in patients with heart failure with preserved ejection fraction [17]. The role of glycocalyx shedding in patients with systolic heart failure and concomitant left ventricular dyssynchrony remains unknown.

Objectives of the current study

1. To confirm activation of glycocalyx shedding in chronic dyssynchronous HFrEF and to identify the clinical determinants of the extent of this putative change with particular regard to severity of heart failure and extent of left ventricular dyssynchrony.
2. To evaluate the potential interactions of glycocalyx shedding with concomitant inflammation, endothelial dysfunction and redox stress, and in particular to determine whether glycocalyx shedding is associated with:
    a. Impaired function of the NO signalling cascade

b. Increased expression of the inflammatory activator, thioredoxin-interacting protein (TXNIP), the expression of which is increased by non-laminar flow [18].

3. To determine whether CRT, possibly by ameliorating non-laminar flow, reduces the extent of glycocalyx shedding.

Methods

Patients with conventional indications for CRT (n=26) were prospectively evaluated before and six months after CRT implant. Patients' quality of life was evaluated using the Minnesota Living with Heart Failure Questionnaire, with higher scores indicating more impaired quality of life.

Echocardiographic measurements

All echocardiographic measurements were performed according to the American Society of Echocardiography and European Association of Cardiovascular Imaging guidelines [19]. A Phillips echocardiogram machine model iE33, 2009, Bothell WA, 98041 USA was used for image acquisition and analyses were performed using Echopac Software Only BT 11 Version 113, 2013 General Electric Co. M-mode echocardiographic analysis was used to assess left ventricular intra-ventricular dyssynchrony. Septal to posterior wall delay (SPWD) was calculated as the time difference between the onset of the QRS to the peak of deformation of the inter-ventricular septum and the left ventricular posterior wall. Although extent of dyssynchrony was analysed as a continuum, SPWD of 130ms or more was considered diagnostic of clinically significant intra-ventricular mechanical dyssynchrony [20]. Left ventricular volumes including end-diastolic, end-systolic and stroke volumes were measured in two dimensions and ejection fraction calculated by the modified Simpson's method in biplane using 2D images.

Radial artery applanation tonometry: determination of augmentation index.

Vascular endothelial function was assessed by changes in augmentation index (AI_X) using radial artery applanation tonometery as previously described [21].

Briefly, patients were first rested in a supine position for 30minutes. Using a commercially available pulse waveform analyser, the SphygmoCor system (AtCor Medical, Sydney, Australia, model CvMS V9) baseline AI_X was computed as the average of three readings. Sublingual nitroglycerin (NTG 300µg) was administered and AI_X re-measured every 5 minutes for twenty minutes. The difference between the lowest value of AI_X with NTG and the baseline AI_X (that is, the maximum change in AI_X with NTG) was utilised as a measure of endothelium- <u>independent</u> NO signalling. Subsequently, 400µg of inhaled salbutamol was administered and measurements were repeated every 5 minutes for twenty minutes. The difference between the lowest AI_X with salbutamol and the baseline (the maximum change in AI_X with salbutamol) represents a measure of endothelium-<u>dependent</u> NO signalling [22]. Using the acquired radial artery waveform, a validated, generalized transfer function was used to generate the corresponding central aortic pressure waveform from which AI_X values were calculated. All measurements were indexed to a heart rate of 75 beats per minute and only high fidelity tracings were used.

Platelet expression of thioredoxin-interacting protein.(TXNIP)

Platelet expression of TXNIP was quantitated by immunofluorescence as previously described [23].

Asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA)

For estimation of asymmetric dimethylarginine and symmetric dimethylarginine concentrations in plasma, 10ml of blood was collected in heparinised vacutainer tubes and immediately put in ice, before centrifugation at 4^0C at 1800g for 15 minutes. Plasma was collected in Eppendorf tubes and stored at -70^0C until analysed. Subsequent determination was performed via high performance liquid chromatography as described by Heresztyn et al [24].

Matrix metalloproteinase-2 and matrix metalloproteinase-9

Matrix metalloproteinase-2 was estimated from blood samples collected in EDTA tubes while matrix metalloproteinase-9 was estimated from blood samples collected in heparinised tubes. For both, collected blood was immediately put on ice and was centrifuged for 15 minutes at 1800g at 4ºC within 30 minutes of collection. Plasma was collected and stored at -70ºC until analysed. Assays were performed with RnD Quantikine quantitative sandwich enzyme-linked immunosorbent assay (ELISA) kits (Minneapolis USA) according to the manufacturer's instructions.

Platelet aggregometry test; responsiveness to NO

Platelet response to NO was assessed in vitro by whole blood impedance aggregometry according to a previously described protocol [25]. Therapy with $P2Y_{12}$ receptor antagonists was temporarily interrupted prior to carrying out the tests in order not to interfere with ADP responses.

***N-Terminal proBNP level*s**

Samples were collected in heparinised tubes and analysed for NT-proBNP levels with the Elecsys proBNP system (Roche diagnostics GmbH, Sandhofer Strasse 116, D-68305

Mannheim, Germany). The Sandwich principle was used according to manufacturer's instructions.

Plasma metanephrine and normetanephrine

Analyses for catecholamine metabolites were performed with fresh samples collected in K_3EDTA bottles and analysed utilizing liquid chromatography-tandem mass spectrometry. The upper limits of the normal range for plasma concentrations were 500 pmol/L for free metanephrine and 900 pmol/L for free normetanephrine.

Measurement of plasma levels of syndecan-1.

For syndecan-1 assay, blood was collected in EDTA tubes and immediately put on ice and centrifuged for 15 minutes at 1800g at 4ºC within 30 minutes of collection. Plasma was collected and stored at -70ºC until analysed. Analysis was performed utilizing Human sCD138 ELISA kit (Diaclone SAS, France version 7, 2015) according to manufacturer's instructions. .

Ethics approval.

The study complied with the *Declaration of Helsinki* and approval for the study was granted by the Ethics and Human Research Committee of The Queen Elizabeth Hospital and all participants provided written informed consent.

Statistical analyses

All data are expressed as mean ± SD unless stated otherwise. Univariate correlations between syndecan-1 levels and endothelial/NO- signalling, echocardiographic parameters and

neurohumoral activation were performed using Pearson correlation coefficients for normally distributed data and Spearman correlation for non-parametric data. Backward stepwise multiple regression analysis was performed utilising parameters with significant univariate correlations with baseline syndecan-1 levels. The interaction of CRT with glycocalyx shedding was assessed by Wilcoxon matched-pairs signed rank test. Apart from the multiple regression analyses performed using SPSS 23 version 11.8.2 (2013 Citrix systems), all other analyses were performed using Prism 6 for Mac OS X version 6.0h October 2015.
A p-value < 0.05 was considered statistically significant.

Results

The baseline characteristics of patients are listed in Table 1. Half of the patients had an ischaemic basis for heart failure, the majority had class III-IV symptomatic status and all had substantial evidence of dyssynchrony both electrically and mechanically. All patients were also extensively treated medically for systolic heart failure prior to CRT implantation. The biochemical and physiological data for this group are summarized in Table 2.
In general, renal function was well preserved, as was platelet response to the NO donor, sodium nitroprusside. Median syndecan-1 levels were clearly elevated beyond the previously reported range for normal subjects (34.1 ± 8.0 ng/ml [26].

Univariate correlates of Syndecan-1 at baseline:

Syndecan-1 levels correlated negatively and significantly with left ventricular ejection fraction ($r=-0.45$, $p=0.02$). There was also a statistically significant positive correlation between syndecan-1 levels and septal to posterior wall delay ($r=0.44$, $p=0.02$) and a statistically significant positive correlation between syndecan-1 and NT-proBNP ($r=0.43$, $p=0.02$). There was a trend towards a significant positive correlation with plasma

normetanephrine (r=0.37, p=0.09). A direct correlation between syndecan-1 levels and degree of impairment of quality of life measured with the Minnesota Living with Heart Failure Questionnaire (with higher values indicating greater impairment) was also observed (r=0.39, p=0.04). These relationships are depicted in Figure 1.

With regard to vascular endothelial function as assessed with applanation tonometry and NO signalling, there was no significant correlation between syndecan-1 levels and baseline augmentation index (r=-0.23, p=NS), nor with change in augmentation index in response to salbutamol(r=-0.12, p=NS). There was also no significant correlation between levels of syndecan-1 and those of asymmetric dimethylarginine (ADMA) (r=0.28, p=0.15), inhibition of ADP-induced platelet aggregation with sodium nitroprusside (r=-0.33, p=0.12), nor with platelet thioredoxin-interacting protein (TXNIP) levels (r=0.22, p=NS). On the other hand, there was a strong and significant positive correlation between syndecan-1 and symmetric dimethylarginine (SDMA), (r=0.54, p=0.003).

Multivariate determinants of syndecan-1 at baseline

Backwards stepwise multiple linear regression analysis was performed utilising parameters that exhibited significant univariate correlations with syndecan-1 levels (ejection fraction, SPWD, SDMA concentrations and NT-pro BNP concentrations, together with baseline eGFR (p=0.10 on univariate analysis).

The results, summarized in Table 3, revealed that baseline SPWD and SDMA concentrations were both directly and significantly related to syndecan-1 levels. Importantly, eGFR was not significantly related to syndecan-1 levels.

Effects of CRT

There was no significant change in syndecan-1 levels between baseline and 6 months post-CRT median (IQR) 55 ng/ml (39.2-75.2) vs 59 ng/ml (31.7-79.6) respectively, p=0.45. There was also no significant correlation between changes in LVEF post-CRT and those in syndecan-1 levels at 6 months, (r=0.31, p=0.13). Similarly, no correlations were found between changes in syndecan-1 levels and other measures of vascular biology and NO signalling including change in baseline augmentation index (r=-0.16, p=NS), change in AI_X with salbutamol (r=-0.35, p=NS), platelet ADP-aggregation inhibition with sodium nitroprusside (r=-0.01, p=NS), ADMA (r= -0.14, p=NS), SDMA (r=0.13, p=NS), nor platelet TXNIP content, (r=0.05, p=NS). In addition, there were no significant correlations between changes in syndecan -1 and NT-proBNP (r=0.04, p=NS), nor with changes in SPWD (r=0.06, p=NS).

Discussion

In this study, we sought to determine whether glycocalyx shedding, as measured by release of syndecan-1 into plasma, plays a major part in the pathophysiology of severe systolic heart failure associated with extensive left ventricular dyssynchrony. The bases for these evaluations were: (1) endothelial glycocalyx shedding may be triggered by inflammatory activation, particularly involving matrix metalloproteinases [27], and (2) in turn may precipitate endothelial dysfunction and abnormal responsiveness of blood vessels to shear stress: all of which have been reported in systolic heart failure [4].

To date, there have been 2 reported clinical studies relevant to the current observations. Tromp et al [17] performed an evaluation of syndecan-1 levels in a large cohort of patients with chronic heart failure, many of whom had preserved ejection fractions. Levels of syndecan-1 were not elevated beyond the normal range (median 20.1ng/ml), and these exhibited correlations on multivariable regression analyses with markers of fibrosis and

remodelling, but not with extent of LV systolic dysfunction. No correlations with extent of dyssynchrony were sought. A Brazilian study [16] of patients with acutely decompensated heart failure (with mean LVEF of 41.5 ±14.4%) revealed far greater mean syndecan-1 levels (approximately 130ng/ml), which were correlated with extent of acute kidney injury and with in-hospital mortality rate. The current study is therefore the first to indicate that chronic systolic heart failure is associated with evidence of increased glycocalyx shedding.

In the current study, univariate analyses suggested that the extent of syndecan-1 elevation was directly related both to the degree of impairment of LVEF and also to the degree of mechanical dyssynchrony. As regards correlations with biochemical/physiological markers, to our surprise there was no correlation between syndecan-1 levels any parameters of integrity of NO signalling, given that in canine models, the integrity of the endothelial glycocalyx layer had been found to flow-mediated NO synthesis[28]. To the best of our knowledge, no previous study had evaluated this putative association in human studies. Therefore it appeared that variability in NO signalling in such patients may have primarily reflected factors such as treatment modalities [29, 30]. In support of this possibility, syndecan-1 levels noted in the Brazilian cohort of patients with acutely decompensated heart failure[16] were much higher than in the currently evaluated patients. In our centre, we have also observed that in patients with acute takotsubo cardiomyopathy, plasma syndecan -1 levels were much higher (97 ± 65ng/ml) [31] than in our cohort of patients with chronic HFrEF, who were already receiving optimal medical treatment.

Syndecan-1 levels also exhibited direct univariate correlations with NT-proBNP and SDMA and this is consistent with greater cardiomyocyte distension and increased inflammation respectively[32, 33].

On multivariate analyses, there were persistent significant associations with degree of mechanical dyssynchrony and with SDMA levels; the latter remained significant after taking eGFR values into account, which is important given the substantial influence of renal function on SDMA clearance [34]. On the other hand, TXNIP expression, which might have been expected to be a direct correlate, given its association with non-laminar flow [18], exhibited no significant association with syndecan-1 levels. Again this may well reflect suppression of TXNIP expression by ACE inhibitor therapy [35].

CRT did not reduce syndecan-1 levels 6 months post procedure in spite of the fact the cohort as a whole exhibited significant improvement in LVEF as well as in clinical symptoms. Thus the lack of effect on syndecan -1 levels may indicate that CRT is relatively ineffective in restoring laminar flow, and/or that the deposition of fibrous tissue leads to ongoing inflammatory activation, which perpetuates glycocalyx shedding

As regards the two significant correlates of syndecan-1 levels on multivariate analyses, (that is, with dyssynchrony and SDMA levels), only association, rather than causation, can be identified definitely at this stage. However, syndecan-1 release appears to engender fibrotic change in tissues [17], and indeed conducting system fibrosis is likely to be critical to development of mechanical dyssynchrony [36]. As regards SDMA, this is generated by protein catabolism and cleared largely, but not entirely, unchanged in the urine. Since it has also been suggested that SDMA may represent a surrogate for glomerular function [34], it is important that its association with syndecan-1 levels was independent of eGFR. Since it has recently emerged that SDMA contributes to inflammatory activation [37], the association with syndecan-1 may reflect the inflammatory origin of glycocalyx shedding, but SDMA may

also contribute to development of myocardial inflammation and fibrosis in this group of patients.

Study Limitations

The study was limited mainly by its small size, with possible risk of Type 2 error, for example, regarding effect of CRT insertion, which might have expected to lower syndecan-1 levels, and also relationship with extent of systolic dysfunction. Importantly, the patients were extensively treated medically and this may have affected findings. Finally, as mentioned earlier, we are unable to draw any definite conclusions regarding cause- and- effect relationships, but it does appear that reducing mechanical dyssynchrony by CRT does not reverse glycocalyx shedding.

Conclusions:

In this study, we demonstrated for the first time that in patients with severe left ventricular systolic dysfunction and concomitant mechanical dyssynchrony, the activation of glycocalyx shedding and that the extent of this process correlated directly with the degree of left ventricular mechanical dyssynchrony. Furthermore, independent of renal function, glycocalyx shedding is also associated with significantly higher plasma levels of the inflammatory marker, SDMA. Larger studies and perhaps with patients with less advanced stages of heart failure are required to shed more light on the possible effects of CRT in patients with systolic heart failure and concomitant left ventricular dyssynchrony.

Clinical Perspectives:

Patients under consideration for cardiac resynchronisation therapy (CRT) usually constitute a group with severe systolic heart failure refractory to conventional pharmacotherapy. In the current study, we provide the first clinical evidence that such CRT candidates suffer from acquired damage to the glycocalyx, or outer layer of endothelial cells. This will cause increased capillary permeability, predisposing towards development of congestion irrespective of severity of hemodynamic changes. Interestingly, glycocalyx damage increased directly with the extent of mechanical dyssynchrony, but was not reversed within 6 months of CRT insertion

Translational outlook:

Glycocalyx 'shedding' is a well-described process activated by pro-inflammatory enzymes such as matrix metalloproteinases. In animal models, inhibition of these enzymes, for example by low dose doxycycline, reverses damage to the glycocalyx, and ameliorates circulatory disturbances. The results of the current study should therefore increase therapeutic focus on peripheral circulatory hemostasis in severe heart failure, and potential for institution of treatments to protect the glycocalyx in such patients.

Conflicts of interest

The authors declare that there was no conflict of interest

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References

1. Krum H, Abraham WT (2009) Heart failure. Lancet 373 :941-955.
2. Candia AM, Villacorta H Jr., Mesquita ET (2007) Immune-inflammatory activation in heart failure. Arq Bras Cardiol 89 :183-190, 201-188.
3. Katz SD, Hryniewicz K, Hriljac I, Balidemaj K, Dimayuga C, Hudaihed A, Yasskiy A (2005) Vascular endothelial dysfunction and mortality risk in patients with chronic heart failure. Circulation 111 :310-314.
4. Ferrari R, Bachetti T, Agnoletti L, Comini L, Curello S (1998) Endothelial function and dysfunction in heart failure. Eur Heart J 19 Suppl G: G41-47.
5. Kolarova H, Ambruzova B, Svihalkova Sindlerova L, Klinke A, Kubala L (2014) Modulation of endothelial glycocalyx structure under inflammatory conditions. Mediators Inflamm 2014: 694312.
6. Mulivor AW, Lipowsky HH (2009) Inhibition of Glycan Shedding and Leukocyte-Endothelial Adhesion in Postcapillary Venules by Suppression of Matrixmetalloprotease Activity with Doxycycline. Microcirculation 16 :657-666.
7. Lipowsky HH (2012) The endothelial glycocalyx as a barrier to leukocyte adhesion and its mediation by extracellular proteases. Ann Biomed Eng 40 :840-848.
8. Cleland JG, Daubert JC, Erdmann E, Freemantle N, Gras D, Kappenberger L, Tavazzi L (2005) The effect of cardiac resynchronization on morbidity and mortality in heart failure. N Engl J Med 352 :1539-1549.
9. Bristow MR, Saxon LA, Boehmer J, Krueger S, Kass DA, De Marco T, Carson P, DiCarlo L, DeMets D, White BG, DeVries DW, Feldman AM (2004) Cardiac-resynchronization therapy with or without an implantable defibrillator in advanced chronic heart failure. N Engl J Med 350 :2140-2150.

10. Pouleur AC, Knappe D, Shah AM, Uno H, Bourgoun M, Foster E, McNitt S, Hall WJ, Zareba W, Goldenberg I, Moss AJ, Pfeffer MA, Solomon SD (2011) Relationship between improvement in left ventricular dyssynchrony and contractile function and clinical outcome with cardiac resynchronization therapy: the MADIT-CRT trial. Eur Heart J 32:1720-1729.

11. Chung ES, Leon AR, Tavazzi L, Sun JP, Nihoyannopoulos P, Merlino J, Abraham WT, Ghio S, Leclercq C, Bax JJ, Yu CM, Gorcsan J 3rd, St John Sutton M, De Sutter J, Murillo J (2008) Results of the Predictors of Response to CRT (PROSPECT) trial. Circulation 117 :2608-2616.

12. Enomoto K, Yamabe H, Toyama K, Matsuzawa Y, Yamamuro M, Uemura T, Morihisa K, Iwashita S, Kaikita K, Sugiyama S, Ogawa H (2011) Improvement effect on endothelial function in patients with congestive heart failure treated with cardiac resynchronization therapy. J Cardiol 58 :69-73.

13. Yufu K, Shinohara T, Ebata Y, Ayabe R, Fukui A, Okada N, Nakagawa M, Takahashi N (2015) Endothelial Function Predicts New Hospitalization due to Heart Failure Following Cardiac Resynchronization Therapy. Pacing Clin Electrophysiol 38 :1260-1266.

14. Warriner DR, Lawford P, Sheridan PJ (2016) Measures of endothelial dysfunction predict response to cardiac resynchronisation therapy. Open Heart 3 : e000391

15. Curry FE, Adamson RH (2012) Endothelial glycocalyx: permeability barrier and mechanosensor. Ann Biomed Eng 40 :828-839.

16. Neves FM, Meneses GC, Sousa NE, Menezes RR, Parahyba MC, Martins AM, Liborio AB (2015) Syndecan-1 in Acute Decompensated Heart Failure--Association With Renal Function and Mortality. Circ J 79 :1511-1519.

17. Tromp J, van der Pol A, Klip IT, de Boer RA, Jaarsma T, van Gilst WH, Voors AA, van Veldhuisen DJ, van der Meer P (2014) Fibrosis marker syndecan-1 and outcome in patients with heart failure with reduced and preserved ejection fraction. Circ Heart Fail 7 :457-462.

18. Wang X-Q, Nigro P, World C, Fujiwara K, Yan C, Berk BC (2012) Thioredoxin Interacting Protein Promotes Endothelial Cell Inflammation in Response to Disturbed Flow by Increasing Leukocyte Adhesion and Repressing Kruppel-Like Factor 2. Circ Res 110 :560-568

19. Lang RM, Badano LP, Mor-Avi V, Afilalo J, Armstrong A, Ernande L, Flachskampf FA, Foster E, Goldstein SA, Kuznetsova T, Lancellotti P, Muraru D, Picard MH, Rietzschel ER, Rudski L, Spencer KT, Tsang W, Voigt JU (2015) Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. J Am Soc Echocardiogr 28:1-39 e14

20. Pitzalis MV, Iacoviello M, Romito R, Massari F, Rizzon B, Luzzi G, Guida P, Andriani A, Mastropasqua F, Rizzon P (2002) Cardiac resynchronization therapy tailored by echocardiographic evaluation of ventricular asynchrony. J Am Coll Cardiol 40:1615-1622

21. Kelly R, Hayward C, Avolio A, O'Rourke M (1989) Noninvasive determination of age-related changes in the human arterial pulse. Circulation 80:1652-1659

22. Hayward CS, Kraidly M, Webb CM, Collins P (2002) Assessment of endothelial function using peripheral waveform analysis: a clinical application. J Am Coll Cardiol 40 :521-528

23. Procter N, Goh V, Mahadevan G, Stewart S, Horowitz J (2016) Platelet Reactivity Is Independent of Left Atrial Wall Deformation in Patients with Atrial Fibrillation. Mediators Inflamm 2016 :9754808.

24. Heresztyn T, Worthley MI, Horowitz JD (2004) Determination of l-arginine and NG,NG- and NG,NG′ -dimethyl-l-arginine in plasma by liquid chromatography as AccQ-Fluor™ fluorescent derivatives. J Chromatogra B Analyt Technol Biomed Life Sci 805 :325-329

25. Chirkov YY, Holmes AS, Chirkova LP, Horowitz JD (1999) Nitrate Resistance In Platelets From Patients With Stable Angina Pectoris. Circulation 100 :129-134

26. Çekiç C, Kırcı A, Vatansever S, Aslan F, Yılmaz HE, Alper E, Arabul M, Sarıtaş Yüksel E, Ünsal B (2015) Serum Syndecan-1 Levels and Its Relationship to Disease Activity in Patients with Crohn's Disease. Gastroenterol Res Pract 2015 :850351.

27. Lipowsky HH, Lescanic A (2013) The Effect of Doxycycline on Shedding of the Glycocalyx Due to Reactive Oxygen Species. Microvasc Res 90:10.1016/j.mvr.2013.1007.1004

28. Mochizuki S, Vink H, Hiramatsu O, Kajita T, Shigeto F, Spaan JA, Kajiya F (2003) Role of hyaluronic acid glycosaminoglycans in shear-induced endothelium-derived nitric oxide release. Am J Physiol Heart Circ Physiol 285 :H722-726

29. Zhang X, Xie Y-W, Nasjletti A, Xu X, Wolin MS, Hintze TH (1997) ACE Inhibitors Promote Nitric Oxide Accumulation to Modulate Myocardial Oxygen Consumption. Circulation 95 :176

30. Kalinowski L, Dobrucki LW, Szczepanska-Konkel M, Jankowski M, Martyniec L, Angielski S, Malinski T (2003) Third-Generation β-Blockers Stimulate Nitric Oxide Release From Endothelial Cells Through ATP Efflux. Circulation 107 :2747

31. Nguyen TH, Liu S, Ong G, Stafford I, Frenneaux M, Horowitz JD (2016) Abstract 11912: Glycocalyx Shedding is Markedly Increased During Acute Phase of Takotsubo Cardiomyopathy. Circulation 134 :A11912

32. Hall C (2005) NT-ProBNP: The Mechanism Behind the Marker. J Card Fail 11 :S81-S83.

33. Schepers E, Glorieux G, Dhondt A, Leybaert L, Vanholder R (2009) Role of symmetric dimethylarginine in vascular damage by increasing ROS via store-operated calcium influx in monocytes. Nephrol Dial Transplant 24 :1429-1435

34. Kielstein JT, Salpeter SR, Bode-Boeger SM, Cooke JP, Fliser D (2006) Symmetric dimethylarginine (SDMA) as endogenous marker of renal function--a meta-analysis. Nephrol Dial Transplant 21 :2446-2451

35. Sverdlov AL, Chan WP, Procter NE, Chirkov YY, Ngo DT, Horowitz JD (2013) Reciprocal regulation of NO signaling and TXNIP expression in humans: impact of aging and ramipril therapy. Int J Cardiol 168 :4624-4630

36. Sugiura M, Okada R, Okawa S, Shimada H (1970) Pathohistological studies on the conduction system in 8 cases of complete left bundle branch block. Jpn Heart J 11 :5-16

37. Schepers E, Barreto DV, Liabeuf S, Glorieux G, Eloot S, Barreto FC, Massy Z, Vanholder R (2011) Symmetric Dimethylarginine as a Proinflammatory Agent in Chronic Kidney Disease. Clin J Am Soc Nephrol 6 :2374-2383

Figure Legends:

Figure 1: Univariate correlates of clinical, echocardiographic and biochemical plasma concentrations of syndecan-1 at baseline:

A: with LVEF

B: with SPWD

C: with NT-proBNP

D: with normetanephrine

E: with QOL score

Figure 2: NO and redox-signalling correlates of plasma syndecan-1 levels at baseline

A: Baseline AIx

B: AIx change with salbutamol

C: Platelet aggregation inhibition with sodium nitroprusside(SNP)

D: with TXNIP

E: with SDMA