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REPORT

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Impaired antioxidant high-density lipoprotein function is associated with premature myocardial infarction [1] (Klaus Distelmaier and Georg Goliasch)

No new evidence on antioxidant high-density lipoprotein (HDL) function has accumulated in patients with premature acute myocardial infarction. However, recent research identified pro-oxidant HDL as a strong and independent predictor of mortality in women with ST-segment elevation myocardial infarction (STEMI) and provides new insights regarding the role of sex hormones, HDL function and clinical events in STEMI patients [2]. Furthermore, there is increasing evidence that oxidative stress dramatically increases during critical illness. Contemporary data in a cohort of 270 intensive care unit (ICU) patients revealed a strong association between survival and antioxidant HDL function [3]. The results of this study suggest that restoration and maintenance of antioxidant HDL capacity might serve as a new therapeutic target in critically ill patients and considering the persistent effect of impaired antioxidant HDL function on clinical outcome, high-risk patients might benefit from specific treatment. Further research is needed to assess which therapeutic interventions might have favourable impact on protective functions of HDL.

A novel protein glycan biomarker and lecithin:cholesterol acyltransferase activity in metabolic syndrome [4] (Robin P. F. Dullaart and Eke G. Gruppen)

Interest with respect to the pathogenic role of glycoproteins in the development of cardiometabolic diseases, inflammation and cancer is growing [1]. In our study, we made use of a newly developed nuclear magnetic spectroscopy-based composite biomarker of systemic inflammation, designated GlycA. GlycA comprehensively reflects the glycosylation state and plasma concentrations of a number of abundant acute-phase proteins [4,5]. Our finding that GlycA levels are higher in subjects with metabolic syndrome [4] has been reinforced in a number of studies, also showing that GlycA correlates positively with obesity measures [5–8]. Interestingly, GlycA has now been demonstrated to predict incident cardiovascular disease, as well as type 2 diabetes mellitus in several population-based cohorts, and this association is at least as strong as that with C-reactive protein [5]. No new evidence has appeared concerning the association of plasma GlycA with lecithin:cholesterol acyltransferase (LCAT) activity, which is increasingly recognized to confer higher cardiovascular risk.

Polymorphisms in CYP-mediated arachidonic acid routes affect the outcome of renal transplantation [9] (Guillermo Gervasini)

More evidence has become available that stresses the relevance of CYP450 genetic variability in the renal transplant outcome. After our 2015 study, we have published a report that points to a polymorphism in CYP4F2, V433M, as an important contributor to the development of post-transplant diabetes mellitus in renal transplant recipients, which may affect the outcome of the process [10]. Of note, CYP4F2 is responsible for 20-hydroxyeicosatetraeonic acid (20-HETE) synthesis, which has been shown to be a mediator of renal cell damage produced by diabetes [11]. In addition, we have also drafted another manuscript, already submitted for publication, on the role of CYP variability in the high cardiovascular (CV) risk observed in renal transplant recipients. In this work, we assess four single nucleotide polymorphisms in the CYP-mediated routes leading to vasoactive eicosanoids (EETs and 20-HETE). Our results show that variability in epoxyeicosatrienoic acids (EET)-synthesizing genes (CYP2C8 and CYP2J2) may modify CV outcomes in renal transplant recipients [12].

Reliability tests and guidelines for B-mode ultrasound assessment of central adiposity [13] (Lee Stoner)

Our EJCI article sought to determine which anatomical structures should be used to ensure optimal reliability of intra-abdominal thickness (IAT) assessments. We found that IAT assessments are most reliable when measurements are made between linea alba and the anterior aspect of the vertebral column. For this previous work, we used a curved array transducer for the scans. This was necessary to compare the reliability of measurements made to various sites. However, the linea alba and the vertebral column can be visualized in the same scan using a linear array transducer. Liner array transducers provide a greater lateral resolution. Therefore, in subsequent work we compared the reliability of IAT assessments made with both curved and linear array transducers [14]. We found that both transducers provided equally reliable measurement of IAT. However, the use of a single linear array transducer does simplify the assessment of central adiposity.

Increased large VLDL particles confer elevated cholesteryl ester transfer in diabetes [15] (Robin P. F. Dullaart)

The constellation of triglyceride-rich very-low-density lipoproteins (VLDL) drives the atherogenic process of

cholesteryl ester transfer, thereby providing a pathogenic mechanism to explain low HDL cholesterol levels in the context of elevated triglycerides [16,17]. The rate of cholesteryl ester transfer is increased in type 2 diabetic patients compared to nondiabetic subjects even when matched for plasma triglyceride levels [18]. Thus, besides VLDL elevations, compositional changes in these lipoproteins probably contribute to enhanced cholesteryl ester transfer in hyperglycaemic individuals as well. No new evidence is available concerning our finding that plasma cholesteryl ester transfer elevations in diabetes are in part attributable increased large VLDL concentrations [15]. Of interest, the cholesteryl ester transfer protein inhibitor, anacetrapib, is effective in raising HDL cholesterol and reducing atherogenic lipoproteins in diabetic subjects [19]. Clinical efficacy of anacetrapib on (recurrent) cardiovascular events is currently tested, and the results will become available shortly.

Prognostic value of RV isovolumic acceleration and tissue strain in moderate HFrEF [20] (Edoardo Sciatti and Enrico Vizzardi)

The prognostic independent role of right ventricular (RV) function in heart failure (HF) is broadly known. We recently demonstrated that RV subclinical impairment assessed by RV strain has a prognostic role in heart failure with reduced ejection fraction (HFrEF) [20] and correlates with RV ejection fraction and volumes calculated with cardiac magnetic resonance (CMR) [21]. Two recent larger studies confirmed our results. RV global peak systolic longitudinal strain (GLS) and free wall longitudinal strain were more accurate to detect subtle RV functional abnormalities in HFrEF than heart failure with preserved ejection fraction (HFpEF), despite normal tricuspid annular plane systolic excursion (TAPSE), S′ at tissue Doppler imaging (TDI) and fractional area change (FAC), and correlated with left ventricular ejection fraction (LVEF) and left ventricular (LV) GLS [22]. Moreover, both RV strain were found associated with all-cause or cardiovascular mortality in univariate and multivariable analyses in HFrEF; independently from several clinical and echocardiographic variables, they were associated with heart transplantation [23]. Finally, in severe HFrEF RV free wall longitudinal strain correlated with myocardial fibrosis detected by histological analysis [24]. To the best of our knowledge, no data have been further published regarding RV isovolumic acceleration (IVA) in HF.

Habitual physical activity is associated with circulating irisin in healthy controls but not in subjects with diabetes mellitus type 2 [25] (Nasser M. Al-Daghri and Shaun Sabico)

In 2015, we observed no significant effects of habitual physical activity in circulating levels of irisin among patients with diabetes mellitus type 2 (DMT2) [25]. Limited investigations overall have been done with respect to the influence of exercise in irisin levels of DMT2 patients and the general population overall, secondary to inconsistent results, heterogeneity of studies and the questionable sensitivity of commercially available kits used in these studies [26]. Nevertheless, promising results on the effects of exercise in irisin levels are seen among nondiabetic adults with varying degrees of obesity [27,28]. It seems likely that the type and length of exercise as well as the degree of obesity are associated more with irisin expression, although the presence of insulin resistance, despite negative results, still cannot be ruled out.

Echocardiographic evaluation in subjects with α1-antitrypsin deficiency [29] (Edoardo Sciatti and Enrico Vizzardi)

We recently studied patients affected by α1-antitrypsin (AAT) deficiency by echocardiography: we found a consistent presence of left and RV diastolic dysfunction [29], mitral valve prolapse [29] and stiffened aortas [30], and we reported a case of idiopathic left ventricular pseudoaneurysm [31]. We hypothesized that cardiovascular involvement with abnormal remodelling is related to chronic inflammation. No new evidence exists about this topic. However, Toldo *et al.* [32] reported the therapeutic role of human AAT infusion (fused to the Fc domain of IgG1) in mice models of anterior myocardial infarction. They demonstrated that the anti-inflammatory effect of AAT has cardioprotective and cytoprotective properties against the ischaemia–reperfusion damage, limiting the infarct size and left ventricular remodelling [32]. The preservation of viable myocardium and systolic function partly support the pathophysiology of cardiovascular involvement in AAT deficiency patients.

Detection of central venous catheter-related bloodstream infections in haematooncological patients [33] (Robert Krause)

No new evidence is available from our group. In our previous study in 3/17 patients, catheter-related bloodstream infections (CRBSI) could not be allocated to certain central venous catheter (CVC) lumina due to inadequate labelling of routine blood culture bottles. Results of CRBSI screening were therefore influenced by lacking correct source information of CRBSI as EDTA blood samples used for screening were drawn from only one catheter lumen/port chamber [33]. Inadequate labelling of blood culture bottles has recently been addressed in a study describing a quality improvement project aiming to increase the occurrence of simultaneous BC drawing with accurate source labelling. It has been shown that only 54% of paired blood

culture bottles obtained from a CVC and a peripheral site were correctly labelled [34]. We therefore conclude that efforts for improving CRBSI diagnostics including new technologies and strategies should also include thorough education of personal involved in care of patients with CVCs.

sST2 levels are associated with all-cause mortality in anticoagulated patients with atrial fibrillation [35] (María Asunción Esteve-Pastor and Francisco Marín)

Soluble suppression of tumorigenicity 2 (sST2) is the soluble form of the receptor protein for interleukin-1 family, and its expression is induced in response to myocardial stress. Our group [35] validated for the first time, the predictive value of sST2 in atrial fibrillation (AF) patients and concluded that sST2 levels had independent predictive value for all-cause mortality. Indeed, several groups have demonstrated the role of sST2 in cardiovascular events [36,37]. The recent clinical guidelines have recommended the use of biomarkers to further refine stroke and bleeding risk in AF patients [38]. AF risk scores based on clinical factors have only moderated ability for predicting adverse events. For that reason, troponin T, GDF-15 or von Willebrand factor are biomarkers that have demonstrated to play a role in risk stratification [39]. However, new analyses are needed to explore the systematic use of sST2 and other biomarkers for clinical decision-making among AF patients.

A novel mutation of the hGR gene causing Chrousos syndrome [40] (Nicolas C. Nicolaides and Evangelia Charmandari)

Chrousos syndrome is a rare endocrinologic condition characterized by partial tissue resistance to glucocorticoids. We and others have investigated the molecular mechanisms of this condition. We proceeded to the functional characterization of the hGRαT556I and demonstrated that the mutant receptor impaired several steps of glucocorticoid cascade, mainly through decreased affinity for the ligand and reduced interaction with coactivators [40]. Velayos *et al.* [41] reported three patients with Chrousos syndrome. The first two patients harboured a novel human glucocorticoid receptor (hGR) mutation (1429C→T), while the third patient had an insertion of four bases between nucleotides 1762 and 1763 forming a premature stop codon at amino acid position 592. Finally, Vitellius and collaborators identified three novel hGR mutations in patients with adrenal incidentalomas and increased glucocorticoid concentrations [42]. The R477S and the Y478C mutations caused reduced DNA binding of the mutant receptors, whereas the L672P led to a defective ligand-binding domain of the receptor [42].

Transient generalized glucocorticoid hypersensitivity [43] (Nicolas C. Nicolaides and Evangelia Charmandari)

In the last 2 years, no progress has been made in the field of pathogenesis of transient generalized glucocorticoid hypersensitivity. Since our published study, nobody in the literature has described a similar case, possibly because the condition is extremely rare.

Sepsis biomarkers in neutropenic systemic inflammatory response syndrome patients on standard care wards [44] (Franz Ratzinger and Heinz Burgmann)

Our study highlighted the inability to identify infection using commonly applied sepsis biomarkers in neutropenic patients with systemic inflammatory response syndrome (SIRS). This finding might be based on the fact that these patients were already highly preselected by applying the SIRS criteria for inclusion. As febrile neutropenia (FN) is more commonly applied as an inclusion criterion in the literature, no new data regarding this specific topic were found in SIRS patients with neutropenia. Our results were confirmed by other working groups in non-neutropenic cancer patients with SIRS or in FN patients with or without proven infection [45,46]. However, procalcitonin (PCT) and lipopolysaccharide-binding protein (LBP) presented a promising performance for identifying microbiologically confirmed bacteraemia, which was recently also observed in other neutropenia settings, including paediatric patients [47–49]. Presepsin (sCD14) might be used in addition to PCT to detect bacteraemia in these patients, which might have a better discriminatory capacity [49].

Effect of vitamins C and E on insulin resistance in diabetes: a meta-analysis study [50] (Ozra Tabatabaei-Malazy and Mostafa Qorbani)

In the last 2 years, three new randomized controlled trials (RCTs) were published in this topic. Among retrieved articles, one RCT [51] assessed the effect of single intake of vitamin E (VE) in 83 diabetic patients, and two RCTs [52,53] evaluated the effect of single intake of vitamin C (VC) in 126 patients with diabetes. Pooling new and previous data for VE or VC supplementation separately, in a new meta-analysis, has not shown a significant effect on homoeostasis model assessment (HOMA) index. Standardized mean difference (SMD) for VE or VC intake were as follows: (−0·041, 95% CI: −0·356 to 0·275, and $P = 0·801$) or (−0·195, 95% CI: −0·435 to 0·045, with $P = 0·112$), respectively, with fixed-effects model.

These new studies have strengthened our previous meta-analysis that sole intake of VC or VE could not improve insulin resistance in diabetics. Pooling new and previous data for VE or VC supplementation separately in a new meta-analysis has not shown any significant improvement in insulin resistance of diabetic patients.

Noninvasive assessment of liver fibrosis in chronic viral hepatitis [54] (Olga Hilda Orasan and Dan Lucian Dumitrascu)

FibroScan, ASAT-to-platelet ratio index (APRI) and fibrosis 4 score (FIB4) are useful noninvasive tests for the evaluation of liver fibrosis (F) stage 4 in patients with chronic viral hepatitis B or C was the main conclusion of our study published in 2015 [54]. We have new data, which reinforces this statement for haemodialysis patients with chronic viral hepatitis. For these patients, we considered FibroScan as standard method for evaluation of F because of the higher risk of complications related to liver puncture biopsy. Hyaluronic acid (HA), APRI, ASAT/ALAT ratio and FIB4 were able to predict F1. HA was the only noninvasive test that could determine F2 and F3. None of the mentioned tests could determine the F4 score. Hyaluronic acid has the highest clinical utility for stage F1 (clinical net benefit 0·22%) and F2 (clinical net benefit 0·18%), but its efficiency to differentiate chronic hepatitis from liver cirrhosis was not demonstrated [55–57].

Involvement of leucocyte/endothelial cell interactions in anorexia nervosa [58] (Victor M Victor and Antonio Hernández-Mijares)

Anorexia nervosa (AN) is a common psychiatric disorder and is related to cardiovascular complications. We have evaluated the effect of AN on metabolic parameters, leucocyte–endothelium interactions, adhesion molecules, proinflammatory cytokines and cardiovascular risk. Oxidative stress was present in patients with AN and lower antioxidants than in controls [59,60]. We evaluated anthropometric and metabolic parameters, interactions between polymorphonuclear neutrophils (PMN) and human umbilical vein endothelial cells (HUVEC), proinflammatory cytokines and cellular adhesion molecules. AN induced a decrease in PMN rolling velocity and an increase in PMN rolling flux and PMN adhesion. Increases in interleukin-6 (IL-6) and tumour necrosis factor alpha (TNF-α), and vascular cell adhesion molecule-1 (VCAM-1) were also observed. This study supports the hypothesis of an association between AN, inflammation and the induction of leucocyte–endothelium interactions. These findings may explain the increased risk of vascular disease among patients with AN. In fact, these results are in agreement with recent studies which suggest that adverse myocardial structural changes occur in AN [61].

Stress and inflammatory biomarkers and symptoms are associated with bioimpedance measures [62] (Charikleia Stefanaki and George P. Chrousos)

In 2014, an association between biomarkers of chronic stress and inflammation, and bioimpedance (BIA) parameters, such as increased fat mass, and decreased skeletal muscle and bone masses measured by an advanced BIA-ACC© apparatus, and medically unexplained symptoms (MUS), was reported in a large population of adult men and women [62]. We suggested that this association could be explained by chronic activation of the stress and inflammatory networks of the organism [62,63]. In 2016, the same BIA method detected a similar association of increased adiposity and early osteosarcopenic features in 18–21 yo men and women, suggesting that chronic stress and inflammation exert their detrimental effects from an early age, already observed in young, seemingly healthy populations [64]. In 2017, a validation study between BIA-ACC© and dual-energy X-ray absorptiometry (DXA) in postmenopausal women showed a remarkable agreement of the two methods and confirmed a marked concordance between fat-free (muscle and bone) and fat masses by both methods. Last, we found strong relations between the presence of MUS and the osteopenia/osteoporosis group [65].

Circulating cardiac biomarkers and postoperative AF in the OPERA trial [66] (Roberto Latini, Deborah Novelli and Serge Masson)

Since the index publication, < 20 papers in English (searching PubMed for 'Post-operative atrial fibrillation' AND 'circulating biomarkers' including two reviews [67]) addressed the issue: besides hypothetical roles played by inflammation, miRNAs (199a and 483-5p in 63 and 28 patients, respectively) [68], oxidative stress [69], and extracellular matrix remodelling, the most frequently studied injury factors, circulating biomarkers that really add to the predictive power of models based on clinical and imaging-based characteristics have not been discovered. Interest in biomarkers as potential therapeutic targets focused on aldosterone, and circulating angiotensin converting enzyme (ACE), but the evidence is inconclusive. Of note, only one negative study was published [70] after the one from OPERA trial. Overall, studies are single-centred, including prospectively no more than 200 patients, a sample size insufficient to reliably assess predictive power of a candidate circulating biomarker [71].

Procedure-related bleeding in elective percutaneous coronary interventions [72] (Gjin Ndrepepa)

Our study that included 9035 patients with stable coronary artery disease (CAD) treated with elective percutaneous coronary intervention (PCI) showed that occurrence of bleeding within 30 days of the procedure was associated with increased risk of 1-year mortality after PCI. These findings suggested that bleeding complications may contribute to suboptimal results of PCI in stable CAD. Since its publication, additional evidence has gathered that occurrence of periprocedural bleeding in patients with CAD is associated with increased risk of subsequent mortality [73–77]. Two recent studies that included 8582 and 5018 patients undergoing PCI showed that bleeding was associated with increased risk of 2-year mortality with the highest risk observed within the first 30 days after PCI [73,74]. Other studies have shown that the survival benefit associated with bleeding avoidance strategies such as the use of radial artery for vascular access or bivalirudin is at least partially explainable by reduced bleeding risk [75–77].

Repeated implantation failure: a new potential treatment option [78] (Antonis Makrigiannakis and Sophia N. Kalantaridou)

Repeated implantation failure (RIF) is a major limiting factor in assisted reproduction. In 2015, we demonstrated that intrauterine administration of autologous corticotrophin-releasing hormone-treated peripheral blood mononuclear cells (CRH-PBMC), prior to fresh blastocyst transfer (day 5), improves clinical pregnancy rates in women with RIF [78]. A decade ago, Yoshioka *et al.* [79] reported that intrauterine administration of human chorionic gonadotropin (HCG)-treated PBMC improves clinical pregnancy rates in this group of infertile women. In 2016, a prospective randomized study showed that HCG-treated PBMC improves clinical pregnancy rates in women with RIF undergoing IVF with frozen/thawed embryo transfer [80]. Finally, in 2017, a cross-sectional study confirmed these findings [81]. We are currently investigating whether CRH-PBMC intrauterine administration, prior to fresh early cleavage stage (day 3) embryo transfer provides similar findings. In all the reported studies, PBMCs were activated either by HCG or CRH; a recent study suggested that untreated PBMC intrauterine administration may also improve clinical outcome in women with RIF [82].

The relationship between renal volume and histology in obese and nonobese kidney donors [83] (Erhan Tatar, Adam Uslu and Huseyin Toz)

In our study, 15% of obese donors – especially those with a tomographic corrected renal volume (cRV) < 195 cm^3 – had a high chronicity score in renal biopsies [83]. At present, there is no new similar study. However after 2015, two large-scale studies investigated the risk of end-stage renal failure (ESRF) in obese kidney donors [84,85]. First study revealed a weak association between ESRF and body mass index (BMI) > 30, with an hazard ratio of 1·16 per increase of 5 above 30 [84]. The second study indicated 1·9-fold higher risk for end-stage renal disease (ESRD) in obese donors. The impact of obesity on ESRF was independent of gender, race and baseline glomerular filtration rate (GFR). As a novel contribution, this study demonstrated increased risk for ESRD among living kidney donors who were overweight (BMI > 27) at baseline [85]. In another study, overweight and obesity at age 17 were strongly associated with ESRF [86]. Thus, in the context of today's literature, overweight and obesity have been shown to increase risk of ESRD in all age groups.

Serum OPN levels are upregulated and predict disability after an ischaemic stroke [87] (Federico Carbone and Fabrizio Montecucco)

In the 2 years following the publication of the study, 'serum osteopontin (OPN) levels are upregulated and predict disability after an ischaemic stroke' [87], no other clinical study specifically focused on our results. Recently, the thrombin-cleaved OPN N-terminal was indicated as potential biomarker of acute atherothrombotic ischaemic stroke, without data on potential prognostic value [88]. Conversely, experimental evidence emphasized the role of OPN in promoting neural stem cells viability via CXCR4 signalling [89]. Further data also indicated OPN as a potential promoter of astrocyte polarization finally leading to re-establishment of the blood–brain barrier after acute ischaemic stroke [90]. Noteworthy, the upregulation of OPN was demonstrated under hyperbaric oxygen preconditioning. In this setting, OPN reduced the expression of interleukin-1β/nuclear factor-κB and increased Akt phosphorylation [91]. Further clinical and basic research studies are warranted to clarify this controversial role of OPN in cerebral ischaemic injury and prognosis.

Bed rest does not induce hypercoagulability [92] (Nandu Goswami and Gerhard Cvirn)

In the original article, we reported that bedrest by itself is not associated with hypercoagulable states in healthy subjects [92].

The evidence has accumulated in the meanwhile that reinforces this statement [93]. We have compared bedrest subjects to two bedrest groups carrying out countermeasures incorporating resistive vibration exercise (RVE), with and without additional supplementation of high-protein diet, to prevent the negative deconditioning of bedrest confinement. We observed that in both groups the haemostatic system shifted towards hypocoagulability [93].

Furthermore, in the original article, we reported that the re-ambulation period is associated with a tendency towards hypercoagulability [92]. The evidence has accumulated in the meanwhile that reinforces this statement, as a tendency towards hypercoagulation was also observed during re-ambulation in the two groups [93] as well as in patients poststroke [94]. These data suggest that orthostatic challenge following bedrest confinement and poststroke might be associated with higher clotting risk.

Influence of bed rest on plasma galanin and adrenomedullin at presyncope [95] (Andreas Roessler and Jerry Joseph Batzel)

In the original article, we reported that bedrest confinement appears to affect adrenomedullin levels as greater increases in adrenomedullin occur at presyncope following bedrest confinement in young healthy males [95]. The evidence that has accumulated in the meanwhile weakens this statement. We recently investigated hormonal responses (adrenomedullin and galanin) during a sit-to-stand protocol in older males and females [96]. We observed that adrenomedullin and galanin levels were similar between gender and did not change during standing. This could be due to the fact that the orthostatic challenge was only for 6 min and none of the participants developed presyncope. We speculated that due to its peripheral vasodilatory effect, the greater levels of adrenomedullin at presyncope following bedrest may have contributed to the reduced orthostatic capacity. We have no new evidence regarding this. To assess the roles these hormones play in orthostatic intolerance, adrenomedullin and galanin should be assessed in participants exhibiting presyncopal symptoms during an orthostatic challenge.

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Appendix Statements made in the Conclusions of the Abstract of original articles published by the European Journal of Clinical Investigation in 2015 and current status for each statement as judged by the authors of each original study.

		Current status for the statement (total number of statements, *N* = 27)				
References	**Statements made in 2015**	**Reinforced (*n* = 17)**	**Modified (*n* = 0)**	**Weakened (*n* = 2)**	**No new evidence (*n* = 10)**	**Other (*n* = 2)**
[1]	Impaired antioxidant function of high-density lipoprotein (HDL) is independently associated with the development of premature acute myocardial infarction. The maintenance of HDL function might evolve into a significant therapeutic target, especially in patients with premature coronary artery disease (CAD).				X	
[4]	A pro-inflammatory glycoprotein biomarker, GlycA, is higher in MetS.	X				
	Higher plasma levels of this glycoprotein biomarker relate to increased lecithin:cholesterol acyltransferase activity in the setting of MetS.					X
[9]	Taken together, these results indicate that variability in the CYP450 genes involved in the synthesis of eicosanoids from arachidonic acid may have a significant impact on graft function and survival in renal transplantation.	X				
[13]	Intra-abdominal thickness assessments to the vertebra were marginally more reliable than those to other structures. While preperitoneal fat thickness assessments were equally reliable for both measurements planes, precise probe placement was easier for the sagittal plane. Based on these findings, guidelines for the reliable measurement of central adiposity using ultrasound are presented.	X				
[15]	Abnormalities in the concentration and composition of large very low density lipoprotein (VLDL) particles are likely to contribute to elevated plasma cholesteryl ester transfer (CET) in Type 2 diabetes mellitus.				X	
[20]	Our study demonstrated a useful prognostic role of right ventricular (RV) strain and isovolumic acceleration, which are parameters of subclinical RV impairment. Patients with low values may benefit from a more aggressive therapy and a closer follow-up.	X				
[25]	This cross-sectional study has shown a weak association of irisin with physical activity levels in healthy controls but not in diabetes mellitus type 2 (DMT2) subjects, suggesting the possibility of discordant regulation in the condition of DMT2.				X	
[29]	In the presence of greater left ventricular mass, a significantly higher incidence of left and right ventricular diastolic dysfunction and mitral valve prolapse occurs in α1-antitrypsin deficiency (AATD) subjects (ZZ genotype). These findings strongly suggest an abnormal remodeling process in cardiac tissue in AATD.	X				

Appendix *Continued*

References	Statements made in 2015	Current status for the statement (total number of statements, *N* = 27)				
		Reinforced (*n* = 17)	Modified (*n* = 0)	Weakened (*n* = 2)	No new evidence (*n* = 10)	Other (*n* = 2)
[33]	Sampling of only one central venous catheter (CVC) lumen/port chamber screening for catheter-related bloodstream infections (CRBSI) in haematooncological patients seems not to be a useful tool for anticipative diagnosis of CRBSI. Reasons for false-negative results might include origin of CRBSIs from the other CVC lumina not sampled for screening, and false-positive results might origin from catheter colonization without subsequent spread of microorganisms into the peripheral bloodstream.				X	
[35]	In an anticoagulated atrial fibrillation (AF) patient's cohort, soluble suppression of tumorigenicity 2 (sST2) levels are an independent predictive factor of all-cause mortality. sST2 levels could be a biomarker used to improve clinical risk assessment in anticoagulated AF patients.	X				
[40]	The natural mutant receptor hGRαH726R impairs multiple steps of glucocorticoid signal transduction, thereby decreasing tissue sensitivity to glucocorticoids.					X*
[43]	Our findings indicate that a transient post-receptor defect, or a virus- or bacterium-encoded molecule, may have enhanced glucocorticoid signal transduction, leading to transient generalized glucocorticoid hypersensitivity and hypo-activation of the hypothalamic-pituitary-adrenal axis.				X	
[44]	In neutropenic systemic inflammatory response syndrome patients, none of the evaluated biomarkers was able to adequately identify infection. Lipopolysaccharide-binding protein and procalcitonin presented a good performance in identifying bacteraemia. Therefore, these markers could be used for screening purposes to increase the pretest probability of blood culture analysis.	X				
[50]	The sole intake of vitamin C, vitamin E or their combination with other antioxidants could not improve insulin resistance in diabetes.	X				
[54]	FibroScan, ASAT to platelet ratio index (APRI) and fibrosis 4 score (FIB4) are useful non-invasive tests for the evaluation of fibrosis stage 4 in patients with chronic hepatitis B and chronic hepatitis C.	X				
[58]	This study supports the hypothesis of an association between anorexia nervosa, inflammation and the induction of leucocyte–endothelium interactions. These findings may explain, in part at least, the increased risk of vascular disease among patients with anorexia nervosa.	X				
[62]	Medically unexplained symptoms (MUS) is an index of chronic stress and inflammation.	X				

Appendix *Continued*

References	Statements made in 2015	Current status for the statement (total number of statements, *N* = 27)				
		Reinforced (*n* = 17)	Modified (*n* = 0)	Weakened (*n* = 2)	No new evidence (*n* = 10)	Other (*n* = 2)
	A novel dual frequency bioimpedance device (BIA-ACC) may provide a useful, bloodless and rapid tool in the clinical setting, distinguishing patients with chronic stress/inflammation from healthy subjects and monitoring their response to treatment.	X				
[66]	Among patients undergoing cardiac surgery, N-terminal pro-B-type natriuretic peptide and high-sensitivity cardiac troponin T are related to clinical and surgical characteristics.	X				
	Among patients undergoing cardiac surgery, N-terminal pro-B-type natriuretic peptide and high-sensitivity cardiac troponin T have different perioperative time courses.				X	
	Among patients undergoing cardiac surgery, N-terminal pro-B-type natriuretic peptide and high-sensitivity cardiac troponin T are not independently associated with risk of postoperative AF.				X	
[72]	In patients with stable CAD undergoing elective percutaneous coronary intervention, the occurrence of bleeding within 30 days of the procedure was associated with increased risk of death at 1 year after percutaneous coronary intervention.	X				
[78]	The current findings support a possible role for the intrauterine administration of autologous corticotrophin-releasing hormone-treated peripheral blood mononuclear cells (PBMCs) in treating women with repeated implantation failure.					X[†]
[83]	In obese cases, decreased renal volume determined by computed tomography is associated with worse renal histology.				X	
[87]	Serum levels of osteopontin, peaked at day 7 after acute ischemic stroke.	X				
	Serum levels of osteopontin predict worse neurological scores.				X	
[92]	Our data indicate that the re-ambulation period is associated with a tendency towards hypercoagulability.	X				
	The results from our study suggest that bed rest by itself is not associated with hypercoagulable states in healthy subjects.	X				

Appendix *Continued*

References	Statements made in 2015	Current status for the statement (total number of statements, $N = 27$)				
		Reinforced ($n = 17$)	Modified ($n = 0$)	Weakened ($n = 2$)	No new evidence ($n = 10$)	Other ($n = 2$)
[95]	Bedrest immobilization appears to affect adrenomedullin levels in that greater increases in adrenomedullin occur at presyncope following bedrest immobilization.			X		
	Due to its peripheral vasculature hypotensive effect, the greater levels of adrenomedullin at presyncope following bedrest immobilization may have contributed to the reduced orthostatic capacity postbedrest.			X		

*These data show that the natural mutant receptors in patients with Chrousos syndrome impair several steps of glucocorticoid signaling depending on the position of the hGR gene mutation.

†The statement is reinforced because intrauterine administration of activated PBMCs, either by human chorionic gonadotropin (HCG) or corticotrophin-releasing hormone (CRH), improves clinical outcome in women with repeated implantation failure [80,81]. It is also modified since intrauterine administration of untreated PBMCs may also improve clinical outcome in women with repeated implantation failure [82].