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1 **Insulin-like Growth Factor Binding Protein 2 predicts mortality risk in heart failure**

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24

25 **Brief title:** IGFBP2, a potential prognostic biomarker for HF

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36

37 **ABSTRACT**

38

39 Background

40 Insulin-like Growth Factor Binding Protein 2 (IGFBP2) showed greater heart failure (HF)  
41 diagnostic accuracy than the “grey zone” B-type natriuretic peptides, and may have  
42 prognostic utility as well.

43

44 Objectives

45 To determine if IGFBP2 provides independent information on cardiovascular mortality in  
46 HF.

47

48 Methods

49 A retrospective study of 870 HF patients from 3 independent international cohorts.  
50 Presentation IGFBP2 plasma levels were measured by ELISA, and patients were followed  
51 from 1 year (Maastricht, Netherlands) to 6 years (Atlanta, GA, USA and Toulouse,  
52 France). Multivariate analysis, Net Reclassification Improvement (NRI) and Integrated  
53 Discrimination Improvement (IDI) were performed in the 3 cohorts. The primary  
54 outcome was cardiovascular mortality.

55

56 Results

57 In multivariate Cox proportional hazards analysis, the highest quartile of IGFBP2 was  
58 associated with mortality in the Maastricht cohort (adjusted hazard ratio 1.69 (95 % CI,  
59 1.18-2.41), p=0.004) and in the combined Atlanta and Toulouse cohorts (adjusted hazard  
60 ratio 2.04 (95%CI, 1.3-3.3), p=0.003). Adding IGFBP2 to a clinical model allowed a  
61 reclassification of adverse outcome risk in the Maastricht cohort (NRI=18.7 % p=0.03;  
62 IDI=3.9 % p=0.02) and with the Atlanta/Toulouse patients (NRI of 40.4% p=0.01, 31,2%  
63 p=0.04, 31.5% p=0,02 and IDI of 2,9% p=0,0005, 3.1% p=0,0005 and 4,2 %, p=0.0005,  
64 for a follow-up of 1, 2 and 3 years, respectively).

65

66 Conclusion

67 In 3 international cohorts, IGFBP2 level is a strong prognostic factor for cardiovascular  
68 mortality in HF, adding information to natriuretic monitoring and usual clinical markers,  
69 that should be further prospectively evaluated for patients’ optimized care.

70 **Key words:** IGFBP2/biomarker / Heart failure / prognosis / natriuretic peptides/  
71 cardiovascular mortality

72

73

74 **Abbreviation list**

75 BNP = B-type natriuretic peptide

76 CI = confidence interval

77 ED = emergency department

78 HF = heart failure

79 IBLOMAVED = Identification of BLOod MARKers for Asymptomatic VEntricular  
80 Dysfunction

81 IGF-1/ IGF-2 = insulin-like growth factor 1 or 2

82 IGFBP2 = insulin-like growth factor binding protein 2

83 IQR = interquartile range or interval

84 LVEF = left ventricular ejection fraction

85 NT-proBNP = N-terminal pro-B type natriuretic peptide

86 NRI = Net Reclassification Index

87 IDI = Integrated Discrimination Improvement

88

## 89 **Introduction**

90 Heart failure (HF) is a considerable social and economic burden in terms of mortality,  
91 morbidity, and associated health care costs. Its current median survival rate is less than 5  
92 years, even in countries with excellent healthcare systems [1]. Despite significant  
93 therapeutic strategy improvement, HF patients have a poor prognosis, with death rates  
94 approximating 40% within a year after a first hospitalization [2]. Biomarkers for the  
95 diagnosis of HF are available, and while the greatest focus has been on natriuretic  
96 peptides [3], several other candidates have been proposed [4, 5]. Unfortunately, most of  
97 the currently available HF biomarkers have significant specificity limitations. This  
98 includes natriuretic peptides, where elevated levels can be associated with non-HF  
99 comorbidities, such that natriuretic peptide concentrations in the “grey zone” frequently  
100 render the test inconclusive [6].

101 Prognostic HF biomarkers may be of utility as an aid to guide therapy, as well as identify  
102 patients at high risk of mortality. However, given the complex pathophysiology of HF,  
103 the wide range of clinical presentations, and the numerous confounding pathologies, it is  
104 not clear that a single biomarker is adequate. Some have suggested a multiple biomarker  
105 strategy may be required to provide a complete picture of clinical status in the HF patient  
106 [7, 8].

107 Early data suggest that measurement of Insulin-like Growth Factor Binding Protein 2  
108 (IGFBP2) may be both a potential diagnostic and prognostic test in the setting of  
109 suspected HF. IGFBP2 is a member of the IGFBP family, and is the second most  
110 abundant in the circulation. IGFBP2 biology was investigated in many studies [9]. This  
111 protein is mainly expressed in the heart and liver, as well as the reproductive and central  
112 nervous systems [10, 11]. However, IGFBP2 mRNA levels were strongly increased in  
113 hearts and not in other organs from a rat model of ischemia induced HF [12]. It manages  
114 transport and bioavailability regulation of Insulin-like Growth Factor 1(IGF-1) [10, 11,

115 13]. In the circulation, IGFBP2 predominately has an inhibitory effect on IGF-1 actions.  
116 This is because of its high affinity binding, as compared to that of IGF-1 for the IGF-1  
117 receptor [14, 15].

118 The inhibition of IGF-1 is mechanistically important in the genesis of HF, as it has been  
119 demonstrated in both human and experimental HF that it is cardioprotective by  
120 downregulating the effects of the renin angiotensin system [16-20]. In fact, a lack of  
121 increase in circulating levels of IGF-1 after acute MI is associated with worse left  
122 ventricular function and worse outcomes [21, 22]. Conversely, elevated IGF-1 is  
123 associated with improved cardiac remodeling and function post-myocardial  
124 infarction. Thus, as elevated IGFBP2 levels may be associated with IGF-1 inhibition that  
125 results in unopposed renin angiotensin effects, elevated IGFBP2 may actually modulate  
126 LV dysfunction [9].

127 That IGFBP2 may be both a diagnostic and prognostic HF marker is further supported by  
128 our recent report in patients presenting with acute dyspnea, where IGFBP2 displayed  
129 greater diagnostic accuracy than “grey zone” B-type natriuretic peptides [12].

130 Thus, in view of the above, our purpose was to determine the ability of IGFBP2 to predict  
131 outcomes using a large cross sectional HF data base. To assess the potential use of  
132 IGFBP2 for HF prognosis, we evaluated 3 international cohorts with varying severity of  
133 disease.

134

## 135 **Methods**

136 We used 3 previously described international HF datasets. This included chronic  
137 hemodynamically stable HF data [23] from the Toulouse University Hospital  
138 (Identification of BLOod Marker of Asymptomatic left Ventricular Dysfunction  
139 (IBLOMAVED)) [23], acute HF data from Maastricht University Medical Center

140 (Netherlands) [24], and chronic hemodynamically outpatient HF data from the Atlanta  
141 Cardiomyopathy Consortium [25]. Cardiovascular mortality was used as endpoint and  
142 determined by reviewing the patient clinical registry.

143 Using -80°C stored plasma from each institution, IGFBP2 levels were determined by  
144 enzyme-linked immunosorbent assay (R&D Systems, Inc., Minneapolis, MN, USA),  
145 performed per the manufacturer's specifications. The detection range for this assay is 62.5  
146 pg/ml to 4000 pg/ml. We defined an appropriate dilution of the plasma (1/800<sup>e</sup>) in order  
147 to monitor IGFBP2 levels in the linear quantitative range of values.

148

#### 149 Chronic Stable HF

150 The IBLOMAVED cohort study (ClinicalTrials.gov NCT01024049) enrolled patients  
151 receiving care at the cardiology department of the University Hospital of Toulouse  
152 (France) from July 2007 to November 2013. This data set consists of 197 stable HF  
153 patients, defined as being without any HF decompensation for at least 3 months, and with  
154 complete survival data [23]. The diagnosis of HF was per clinical observations,  
155 transthoracic echocardiography (TTE), and BNP monitoring.

156 This research protocol was approved by the institution's human research (COSSEC) and  
157 regional ethics committee (Comité de Protection des Personnes (CPP) # DC 2008-452)  
158 and complied with the Declaration of Helsinki. Written informed consent was obtained  
159 from all participants or their legally authorized representatives.

160

#### 161 Emergency Department (ED) HF

162 Maastricht University Medical Center (Netherlands) prospectively enrolled 678 patients  
163 presenting with dyspnea to the emergency department from June 2007 to October 2009.  
164 Of these, 367 had acute HF and formed our study population. HF was diagnosed by a

165 senior cardiologist [24], and patient characteristics were determined by clinical chart  
166 review. Left ventricular ejection fraction (LVEF) was obtained from echography, when  
167 available, within a range of 1 year before to 1 month after presentation. All patients were  
168 followed for 1 year. Follow-up data were obtained via chart review and the patient's  
169 general practitioner, or by inquiry of the municipal register. The primary outcome  
170 measure was 1-year cardiovascular mortality. All investigational procedures were  
171 approved by the institutional review board (Medical Ethical Committee, Maastricht  
172 University Medical Center) and complied with the Declaration of Helsinki.

173

#### 174 Outpatient HF

175 The Atlanta Cardiomyopathy Consortium (TACC) prospective cohort study [25] enrolled  
176 335 patients from November 2007 to February 2014, at 3 university-affiliated hospitals in  
177 the greater metropolitan Atlanta area (ClinicalTrials.gov, NCT00599014). All patients  
178 underwent history surveys, physical examination, and collection of blood samples at  
179 baseline. Cardiovascular mortality data were collected through medical record review,  
180 family members, and U.S. social security death index query. Of 335 patients, 306 (91.3%)  
181 had plasma available for IGFBP2 assessment. The primary outcome was any major  
182 clinical event, defined as a composite of death, heart transplantation, or left ventricular  
183 assist device placement. The Institutional Review Board has approved the study. Written  
184 informed consent was obtained from all participants or their legally authorized  
185 representatives.

186

#### 187 **Statistical methods and identification of biomarkers**

188 Evaluation of normally distributed continuous variables was by Student's t-test, otherwise  
189 a Mann-Whitney rank sum test was used. Evaluation of categorical variables was by chi  
190 square test. We tested the survival of HF patients, stratified by IGFBP2 level, using



191 Kaplan Meier curves and multivariate Cox regression analyses, performed with Medcalc  
192 software (version 14.8.1, Medcalc software bva, Belgium). Poisson regression analysis  
193 was performed using R computation language with “Modern Applied Statistics with S”  
194 package version 7.3-35. Net Reclassification Improvement (NRI) and Integrated  
195 Discriminant Improvement (IDI) analyses for improvement in predictive accuracy for  
196 mortality by the addition of IGFBP2 to clinical models were performed using R software  
197 and the “survIDINRI” package version 1.1-1. Clinical risk factors for cardiovascular  
198 death were chosen as age, gender, diabetes status and creatinine and age, gender, ALT,  
199 hs-CRP, creatinine to include relevant parameters in the risk prediction model for  
200 hemodynamically stable or acute HF, respectively.

## 201 **Results**

202 Demographic and clinical characteristics of the total dataset are presented in **Table 1**.  
203 While the Toulouse and Atlanta chronic stable HF had a number of similarities, the ED  
204 cohort had several important differences. ED patients had more than double median  
205 IGFBP2 levels compared to the chronic stable and outpatient cohorts; 393 vs 165 and 172  
206 ng/mL, respectively. ED patients were also older (median age 78, vs 60 and 57 years) and  
207 more likely women (46%, vs 21 and 36%) than the chronic stable HF and  
208 cardiomyopathy clinic cohort patients, respectively. ED patients also had less guideline  
209 recommended care in every single category, with the exception of angiotensin receptor  
210 blocker administration, for which patients were 50% more likely to receive this than in  
211 the chronic stable HF cohort.

212 Chronic stable HF: The median follow up of the 197 chronic stable HF IBLOMAVED  
213 patients was 3.6 years (43 months) [IQR, 2.9-14.2;(35-50 months)]. Of the 197, 57  
214 (29%) died from a cardiac cause and 140 (71%) were end-of-study censored. Overall, the  
215 median IGFBP2 level was 165 ng/ml [IQR, 75-391], and levels were higher in patients  
216 who died compared to survivors; 289 ng/ml (95% CI, 206 – 444) vs 126 ng/ml (95% CI,  
217 114 – 165), respectively (p<0.001). Patients (n=49) with IGFBP2 in the highest quartile,

218 i.e. >391 ng/ml, had a much higher 6 year mortality, Hazard Ratio (HR) = 6.25 (95% CI,  
219 2.73-7.93),  $p < 0.0001$ , and a median survival time of 1.7 years (95% CI, 0.6 – 3.3). This  
220 compared to all other patients (n=148) whose mean survival was 5.1 years (95% CI, 4.7 –  
221 5.3). Separate analysis of Atlanta and Toulouse cohorts provided the same results for  
222 cardiovascular mortality with median value IGFBP2 concentration of 172 and 165 ng/mL,  
223 respectively (**Supplementary Figure 1**).

#### 224 Combined chronic HF patients cohorts (Atlanta and Toulouse IBLOMAVED)

225 In this combined chronic HF patient cohort, admission IGFBP2 level was associated with  
226 2 years cardiovascular mortality across the concentration range (**Figure 1A**), although  
227 hazard ratios were non-significant in the lowest 3 quartiles (HR=1.0, 1.2 and 1.8,  
228 respectively,  $p > 0.05$ ). However, the fourth quartile was associated with 35 % 2 years  
229 mortality risk (HR=3.3 (95% CI, 1.4-4.7),  $p = 0.02$ ).

230 Multivariate Cox proportional hazards regression analysis adjusted for age, gender,  
231 diabetes status, creatinine, IGFBP2 and BNP levels dichotomized by highest quartile,  
232 showed that IGFBP2 is an independent predictor of poor outcome (HR=2.04 (95%CI,  
233 1.3-3.3),  $p = 0.003$ ) (**Table 2A**).

234 Interaction Forest plot provides a global view of interactions between IGFBP2 plasmatic  
235 level and the heart failure patient's clinical characteristics value. Interaction Forest plot  
236 representation and analysis of the interaction between IGFBP2 prognostic value and  
237 clinical data (sodium, diabetes, creatinine, BNP, gender, age) did not show evidence of  
238 heterogeneity ( $p$  values  $> 0.05$ ). This indicates that even with fluctuation of these clinical  
239 parameters, IGFBP2 remains a prognostic biomarker.

240 (**Supplementary figure 2A and Supplementary Table 1**).

241 Addition of IGFBP2 dichotomized by upper quartile to a clinical model (age, gender,  
242 diabetes status and creatinine) improved reclassification of HF patients according to

243 survival over 1 to 3 years of follow-up (**Table 2B**). Addition of IGFBP2 to the clinical  
244 model containing BNP allowed a better reclassification at 1 year (NRI=40.4% p=0.0005),  
245 2 years (NRI=25.9%, p=0.04) and 3 years (NRI=28.5% p=0.01) of follow-up. IDI values  
246 ranged from 1.6 % (p=0.06) at one year to 3% (p=0.01) at 3 years of follow-up in the  
247 clinical model by addition of BNP. However, addition of IGFBP2 to the clinical model  
248 led to an IDI of 2.9 to 4.2 % (p=0.0005). Combined integration of BNP and IGFBP2 with  
249 the clinical model did not improve IDI.

250 ED patients: In the ED cohort, admission IGFBP2 level was associated with  
251 cardiovascular mortality across the concentration range (Log-rank for test for trend, p =  
252  $10^{-5}$ )(**Figure 1B**). IGFBP2 level in the third and the fourth quartile was associated with a  
253 significant increase in cardiovascular mortality over 1 year of follow up; HR=2.2  
254 (95%CI, 1.4-3.5), p=0.001 and HR=3.3 (95%CI, 2.2-5.1) p<0.0001, respectively.

255 The median survival for a patient with IGFBP2 level in the highest quartile (>551 ng/ml)  
256 was 191 days (95% CI, 96-286), whereas median survival for a patient with a  
257 concentration below 240 ng/ml exceeded 1 year. Results of multivariate Cox proportional  
258 hazards regression analyses adjusted for age, gender, levels of ALT, hs-CRP, creatinine,  
259 sodium, and IGFBP2 and NT-proBNP levels dichotomized by upper quartile, are  
260 presented in **Table 3A**. Creatinine and IGFBP2 in the highest quartile (>551 ng/ml) were  
261 independent predictors of poor outcome, HR = 1.02(95% CI, 1.01-1.03), p=0.005 and  
262 HR=1.69 (95% CI, 1.2-2.4), p=0.004 respectively. NT-proBNP was not associated with 1  
263 year mortality.

264 Forest plot representation analysis of the interaction between IGFBP2 prognostic value  
265 and key clinical data (sodium, creatinine, hs-CRP, ALT, NT-proBNP, gender and age)  
266 did not show evidence of heterogeneity with p values over 0.05 (**Supplementary figure**  
267 **2B and Supplementary Table 2**).

268 Adding IGFBP2 levels to a clinical model (including age, gender, sodium, ALT, hs-CRP,  
269 creatinine) allowed a significant reclassification of HF patients for 1 year survival  
270 (NRI=18.7% p=0.03), even when NT-proBNP was included in the clinical model  
271 (NRI=19.1% p=0.02). NT-proBNP added to the clinical model didn't reclassify patients  
272 (NRI=6.2% p=0.458) nor did provide a significant IDI. Furthermore, addition of IGFBP2  
273 in the clinical model led to an IDI of 3.9 % (p=0.02) which did not remain significant by  
274 adding NT-proBNP (IDI=3.8 % p=0.05) analysis (**Table 3B**).

275

## 276 **Discussion**

277 In a mixed cohort of 870 HF patients with different acuity profiles, ranging from the  
278 emergency department acute decompensated HF patient, to stable chronic HF patients, we  
279 describe the relationship between elevated IGFBP2 levels and the prognosis for both  
280 acute and long-term outcomes. The association between IGFBP2 levels and prognosis  
281 was independent of, and complementary to the standard of care prognostic natriuretic  
282 peptide markers measured in each cohort.

283 This international 3 cohorts design results in broad variations of underlying standard of  
284 care treatments that are likely to result in confounders between outcomes and IGFBP2  
285 levels in and of themselves. However, this variation and our consistent results provide  
286 reassurance that our findings are the consequence of underlying pathology and not simply  
287 treatment effects. This supports the utility of IGFBP2 in that its prognostic capacity  
288 remains valid despite potentially confounding clinical situations, treatments, and range of  
289 acuity. Finally, the potential value of IGFBP2 must be considered in light of prior work  
290 [12] that has shown that IGFBP2 has better diagnostic discrimination for HF than  
291 natriuretic peptides. Natriuretic peptides are in most studies adding prognostic value to  
292 clinical models. However, use of natriuretic peptides for clinical improvement needs  
293 further studies [26, 27]. Thus, the combination of both diagnostic and prognostic

294 capacities that are equivalent or even superior to natriuretic peptides suggests that IGFBP2  
295 has potential utility in altering treatment of patients pending results of future trials.

296 In this analysis, it must be considered that the median value of IGFBP2 was different in  
297 the chronic and acute HF cohorts. In ED patients, a population most likely to have the  
298 greatest acuity at presentation, the median level was the highest (393 ng/ml). This  
299 compares to the lower and similar levels in the chronic stable and outpatient  
300 cardiomyopathy clinic patients (median levels of 165 and 172 ng/ml, respectively). These  
301 findings support the concept that IGFBP2 levels are influenced by the clinical  
302 characteristics of the cohorts. Ultimately our results are consistent with the expectation  
303 that an accurate diagnostic and prognostic biomarker would be affected by the severity of  
304 illness of the evaluated population.

305 It is known that IGFBP2 is expressed in renal tissue [28]. Moreover, few studies have  
306 correlated IGFBP2 plasmatic levels to renal function in pathological conditions, like  
307 diabetes [29] or chronic renal failure [30]. However, in Toulouse University Hospital and  
308 Atlanta Outpatient HF cohorts IGFBP2 was a strong mortality predictor (HR=2.14,  
309 p=0.002) and creatinine level was not (HR=0.99, p=0.49; Table 2 and Supplementary  
310 Figure 2A). Furthermore in Maastricht ED cohort (Table 3 and Supplementary Figure  
311 2B), IGFBP2 displayed a elevated HR (HR=1.64, p=0.006) whereas creatinine level was  
312 not providing a strong HR but significant (HR=1.02; p=0.006). Therefore, if renal  
313 function influence on IGFBP2 levels cannot be excluded, in our cohort the renal failure  
314 patients were excluded from recruitment, the pronostic value of IGFBP2 remains strong  
315 in HF patients. Furthermore, IGFBP2 prognostic value tested in interaction Forest plot  
316 and calculation of the interaction p-value did not show evidence of heterogeneity. A  
317 recent meta-analysis of IGFBP2s plasma levels in 16,024 men from 22 studies showed that  
318 blood IGFBP2 levels fluctuate in non HF patients [31] but remain very low compared to  
319 patients with acute or chronic heart failure. We observed that IGFBP2 plasma  
320 concentration can be as low a few ng/ml (5-10 ng/ml) in healthy individuals and can rise

321 up to over 3000 ng/ml in very severe heart failure patients [12]. These observations are in  
322 favor of a possible use of IGFBP2 in the clinic.

323 It is well known that many of the currently available HF biomarkers display, to some  
324 extent, prognostic capacities. For instance, in a meta-analysis of 19 studies evaluating  
325 natriuretic peptides for death or cardiovascular events, BNP, NT-proBNP, and ANP were  
326 shown to have prognostic value in HF. They report that a BNP increase of 100 pg/ml in  
327 HF was associated with a 35% elevation of the relative risk of death [32]. A more recent  
328 meta-analysis of seventy-nine studies, with a follow-up interval from 14 days to 7 years,  
329 showed that higher levels of BNP or NT-proBNP on admission were associated with  
330 greater risk of mortality, and that a post-admission decrease was associated with a better  
331 outcome [33]. In our analysis, natriuretic peptides were predictor of mortality in the  
332 chronic heart failure cohorts (Atlanta and Toulouse) but not in the Maastricht Emergency  
333 Department cohort (HR = 1.31 (95% CI 0.91-1.89); p=0.144). The reason may be that this  
334 cohort contained only 51 % of patients with a left ventricular ejection fraction below 40%  
335 (as is common in the ED population), whereas the chronic heart failure cohorts had higher  
336 rates of reduced EF (76 % and 60 % for Toulouse and Atlanta, respectively). This may  
337 explain that, despite a rise in NT-proBNP for some patients, NT-proBNP levels were  
338 globally lowered for this cohort and therefore its prognostic value was impaired.

339 These observations lead us to propose that future trials include serial analysis of  
340 biomarker levels and direct comparison of available biomarkers and IGFBP2 for outcome  
341 prediction. An evaluation of the serial assessment of IGFBP2 is needed to determine if  
342 level change occurs with alterations in clinical status[23, 34].

343 [35][36][37][38][39]In the present study IGFBP2 provides a very high NRI (20 to 40 %  
344 range) and rather high IDI (2.9 to 4.2 %), for both acute and chronic heart failure patients.  
345 The high NRI and IDI confirm high clinical relevance of IGFBP2 for prognosis and

346 supports the hypothesis that patients with high IGFBP2 levels could have improved and  
347 adapted care to avoid their predicted poor outcomes.

348 IGFBP2 has essentially a role by its in vivo regulation of the activity of Insulin Growth  
349 Factor 1 (IGF1), by interacting with membrane receptors, such as the PTP  $\beta$  receptor,  
350 whose inhibition by IGFBP2 prevents inactivation of the pathway PI3K / Akt, stimulated  
351 by IGF1, resulting in a pro-proliferative effect and anti-apoptotic [34]. On the other hand  
352 higher IGFBP2 concentrations results in inhibition of this pathway [35, 36].

353 IGF1 is involved in left ventricular remodeling, associated with an increase in left  
354 ventricular end-diastolic volume and a resulting increase in systolic ejection volume of  
355 the cardiac output when injected into the rat or man [37, 38]. Moreover, IGF1 addition  
356 showed in vitro the proliferation of cardiomyocytes favoring this left ventricular  
357 hypertrophy [39].

358 Finally, IGFBP2 has an activity unrelated to the modulation of IGF, as positive regulator  
359 of gene expression, since its nuclear translocation under oxidative stress led to a parallel  
360 increase in VEGF expression [40]. As a consequence, IGFBP2 has a pro-proliferative and  
361 anti-apoptotic effects mediated by VEGF.

362 IGFBP2 may act as a modulator of left ventricular remodeling, promoting beneficial  
363 remodeling for left ventricular function, where sustained activation over time of IGF1  
364 could switch this remodeling mechanism to a deleterious mode, with too much dilation  
365 altering left ventricular contractility according to Frank-Starling's law. IGFBP2's role in  
366 cardiac remodeling needs to be further investigated in animal models of heart dysfunction  
367 and failure.

368 This study is not without limitations. As a retrospective analysis, our findings are limited  
369 to hypothesis generating associations. Second, as clinicians were blinded to the IGFBP2  
370 results, no statement on how IGFBP2 levels could affect outcomes by changes in therapy  
371 can be considered. Third, since we used retrospective cohorts with cardiovascular

372 mortality records only we could not provide all-cause mortality influence. Fourth,  
373 hospitalization prediction was not studied in this work h. Fifth, we did not compare  
374 IGFBP2 prognosis value to the one provided by other biomarkers such as cardiac  
375 troponins or sST2. However, prognosis value comparison of natriuretic peptides with  
376 these biomarkers was described previously [41].

377 Finally, further prospective multicenter studies investigating the benefit of IGFBP2  
378 measurement on patients outcome and defining the consequence of confounding  
379 conditions for IGFBP2 levels are needed before IGFBP2 can be uniformly adopted in the  
380 clinic.

### 381 **Conclusions:**

382 IGFBP2 provides strong prognostic information for cardiovascular mortality risk in  
383 patients with HF. Further studies are needed to demonstrate that knowledge of IGFBP2  
384 levels during patient care will result in improved outcomes.

385

### 386 **REFERENCES**

- 387 [1] Maison P, Desamericq G, Hemery F, Elie N, Del'volgo A, Dubois-Rande JL, et al.  
388 Relationship between recommended chronic heart failure treatments and mortality over  
389 8 years in real-world conditions: a pharmacoepidemiological study. *Eur J Clin Pharmacol.*  
390 2013;69:901-8.
- 391 [2] Gheorghide M, Vaduganathan M, Fonarow GC, Bonow RO. Rehospitalization for  
392 heart failure: problems and perspectives. *J Am Coll Cardiol.* 2013;61:391-403.
- 393 [3] Adams KF, Jr., Felker GM, Fraij G, Patterson JH, O'Connor CM. Biomarker guided  
394 therapy for heart failure: focus on natriuretic peptides. *Heart Fail Rev.* 2010;15:351-70.
- 395 [4] Rocchiccioli JP, McMurray JJ, Dominiczak AF. Biomarkers in heart failure: a clinical  
396 review. *Heart Fail Rev.* 2010;15:251-73.
- 397 [5] de Boer RA, Daniels LB, Maisel AS, Januzzi JL, Jr. State of the Art: Newer biomarkers in  
398 heart failure. *Eur J Heart Fail.* 2015;17:559-69.
- 399 [6] Coste J, Pouchot J. A grey zone for quantitative diagnostic and screening tests. *Int J*  
400 *Epidemiol.* 2003;32:304-13.
- 401 [7] Richter B, Koller L, Hohensinner PJ, Zorn G, Brekalo M, Berger R, et al. A multi-  
402 biomarker risk score improves prediction of long-term mortality in patients with  
403 advanced heart failure. *Int J Cardiol.* 2013;168:1251-7.
- 404 [8] Apple FS, Murakami MM, Pearce LA, Herzog CA. Multi-biomarker risk stratification of  
405 N-terminal pro-B-type natriuretic peptide, high-sensitivity C-reactive protein, and  
406 cardiac troponin T and I in end-stage renal disease for all-cause death. *Clin Chem.*  
407 2004;50:2279-85.



408 [9] Haywood NJ, Slater TA, Matthews CJ, Wheatcroft SB. The insulin like growth factor  
409 and binding protein family: Novel therapeutic targets in obesity & diabetes. *Mol Metab.*  
410 2019;19:86-96.

411 [10] Kang HS, Kim MY, Kim SJ, Lee JH, Kim YD, Seo YK, et al. Regulation of IGFBP-2  
412 expression during fasting. *Biochem J.* 2015;467:453-60.

413 [11] Wheatcroft SB, Kearney MT. IGF-dependent and IGF-independent actions of IGF-  
414 binding protein-1 and -2: implications for metabolic homeostasis. *Trends Endocrinol*  
415 *Metab.* 2009;20:153-62.

416 [12] Berry M, Galinier M, Delmas C, Fournier P, Desmoulin F, Turkieh A, et al. Proteomics  
417 analysis reveals IGF2BP2 as a candidate diagnostic biomarker for heart failure. *IJC*  
418 *Metabolic & Endocrine.* 2015;6:5–12.

419 [13] Ricort JM. Insulin-like growth factor binding protein (IGFBP) signalling. *Growth*  
420 *Horm IGF Res.* 2004;14:277-86.

421 [14] Pintar JE, Schuller A, Cerro JA, Czick M, Grewal A, Green B. Genetic ablation of  
422 IGF2BP2 suggests functional redundancy in the IGF2BP family. *Prog Growth Factor Res.*  
423 1995;6:437-45.

424 [15] Hedbacker K, Birsoy K, Wysocki RW, Asilmaz E, Ahima RS, Farooqi IS, et al.  
425 Antidiabetic effects of IGF2BP2, a leptin-regulated gene. *Cell Metab.* 2010;11:11-22.

426 [16] Duerr RL, Huang S, Miraliakbar HR, Clark R, Chien KR, Ross J, Jr. Insulin-like growth  
427 factor-1 enhances ventricular hypertrophy and function during the onset of  
428 experimental cardiac failure. *J Clin Invest.* 1995;95:619-27.

429 [17] Jin H, Yang R, Gillett N, Clark RG, Ko A, Paoni NF. Beneficial effects of growth  
430 hormone and insulin-like growth factor-1 in experimental heart failure in rats treated  
431 with chronic ACE inhibition. *J Cardiovasc Pharmacol.* 1995;26:420-5.

432 [18] Yang R, Bunting S, Gillett N, Clark R, Jin H. Growth hormone improves cardiac  
433 performance in experimental heart failure. *Circulation.* 1995;92:262-7.

434 [19] Fazio S, Sabatini D, Capaldo B, Vigorito C, Giordano A, Guida R, et al. A preliminary  
435 study of growth hormone in the treatment of dilated cardiomyopathy. *N Engl J Med.*  
436 1996;334:809-14.

437 [20] Genth-Zotz S, Zotz R, Geil S, Voigtlander T, Meyer J, Darius H. Recombinant growth  
438 hormone therapy in patients with ischemic cardiomyopathy : effects on hemodynamics,  
439 left ventricular function, and cardiopulmonary exercise capacity. *Circulation.*  
440 1999;99:18-21.

441 [21] Lee WL, Chen JW, Ting CT, Lin SJ, Wang PH. Changes of the insulin-like growth factor  
442 I system during acute myocardial infarction: implications on left ventricular remodeling.  
443 *J Clin Endocrinol Metab.* 1999;84:1575-81.

444 [22] Conti E, Andreotti F, Sciahbasi A, Riccardi P, Marra G, Menini E, et al. Markedly  
445 reduced insulin-like growth factor-1 in the acute phase of myocardial infarction. *J Am*  
446 *Coll Cardiol.* 2001;38:26-32.

447 [23] Koukoui F, Desmoulin F, Galinier M, Barutaut M, Caubere C, Evaristi MF, et al. The  
448 prognostic value of plasma galectin-3 in chronic heart failure patients is maintained  
449 when treated with mineralocorticoid receptor antagonists. *PLoS One.*  
450 2015;10:e0119160.

451 [24] Eurlings LW, Sanders-van Wijk S, van Kimmenade R, Osinski A, van Helmond L,  
452 Vallinga M, et al. Multimarker strategy for short-term risk assessment in patients with  
453 dyspnea in the emergency department: the MARKED (Multi mARKer Emergency  
454 Dyspnea)-risk score. *J Am Coll Cardiol.* 2012;60:1668-77.

455 [25] Marti CN, Georgiopoulou VV, Giamouzis G, Cole RT, Deka A, Tang WH, et al. Patient-  
456 reported selective adherence to heart failure self-care recommendations: a prospective  
457 cohort study: the Atlanta Cardiomyopathy Consortium. *Congest Heart Fail.* 2013;19:16-  
458 24.

459 [26] Don-Wauchope AC, Santaguida PL, Oremus M, McKelvie R, Ali U, Brown JA, et al.  
460 Incremental predictive value of natriuretic peptides for prognosis in the chronic stable  
461 heart failure population: a systematic review. *Heart Fail Rev.* 2014;19:521-40.  
462 [27] Pufulete M, Maishman R, Dabner L, Higgins JPT, Rogers CA, Dayer M, et al. B-type  
463 natriuretic peptide-guided therapy for heart failure (HF): a systematic review and meta-  
464 analysis of individual participant data (IPD) and aggregate data. *Syst Rev.* 2018;7:112.  
465 [28] Fujinaka H, Katsuyama K, Yamamoto K, Nameta M, Yoshida Y, Yaoita E, et al.  
466 Expression and localization of insulin-like growth factor binding proteins in normal and  
467 proteinuric kidney glomeruli. *Nephrology (Carlton).* 2010;15:700-9.  
468 [29] Narayanan RP, Fu B, Heald AH, Siddals KW, Oliver RL, Hudson JE, et al. IGFBP2 is a  
469 biomarker for predicting longitudinal deterioration in renal function in type 2 diabetes.  
470 *Endocr Connect.* 2012;1:95-102.  
471 [30] Fouque D, Le Bouc Y, Laville M, Combarrous F, Joly MO, Raton P, et al. Insulin-like  
472 growth factor-1 and its binding proteins during a low-protein diet in chronic renal  
473 failure. *J Am Soc Nephrol.* 1995;6:1427-33.  
474 [31] Watts EL, Perez-Cornago A, Appleby PN, Albanes D, Ardanaz E, Black A, et al. The  
475 associations of anthropometric, behavioural and sociodemographic factors with  
476 circulating concentrations of IGF-I, IGF-II, IGFBP-1, IGFBP-2 and IGFBP-3 in a pooled  
477 analysis of 16,024 men from 22 studies. *Int J Cancer.* 2019.  
478 [32] Doust JA, Pietrzak E, Dobson A, Glasziou P. How well does B-type natriuretic peptide  
479 predict death and cardiac events in patients with heart failure: systematic review. *BMJ.*  
480 2005;330:625.  
481 [33] Santaguida PL, Don-Wauchope AC, Oremus M, McKelvie R, Ali U, Hill SA, et al. BNP  
482 and NT-proBNP as prognostic markers in persons with acute decompensated heart  
483 failure: a systematic review. *Heart Fail Rev.* 2014;19:453-70.  
484 [34] Sharples AP, Al-Shanti N, Hughes DC, Lewis MP, Stewart CE. The role of insulin-like-  
485 growth factor binding protein 2 (IGFBP2) and phosphatase and tensin homologue (PTEN)  
486 in the regulation of myoblast differentiation and hypertrophy. *Growth Horm IGF Res.*  
487 2013;23:53-61.  
488 [35] Hoeflich A, Nedbal S, Blum WF, Erhard M, Lahm H, Brem G, et al. Growth inhibition  
489 in giant growth hormone transgenic mice by overexpression of insulin-like growth  
490 factor-binding protein-2. *Endocrinology.* 2001;142:1889-98.  
491 [36] Shen X, Xi G, Maile LA, Wai C, Rosen CJ, Clemmons DR. Insulin-like growth factor  
492 (IGF) binding protein 2 functions coordinately with receptor protein tyrosine  
493 phosphatase beta and the IGF-I receptor to regulate IGF-I-stimulated signaling. *Mol Cell*  
494 *Biol.* 2012;32:4116-30.  
495 [37] Donath MY, Sutsch G, Yan XW, Piva B, Brunner HP, Glatz Y, et al. Acute  
496 cardiovascular effects of insulin-like growth factor I in patients with chronic heart failure.  
497 *J Clin Endocrinol Metab.* 1998;83:3177-83.  
498 [38] Osterziel KJ, Ranke MB, Strohm O, Dietz R. The somatotrophic system in patients  
499 with dilated cardiomyopathy: relation of insulin-like growth factor-1 and its alterations  
500 during growth hormone therapy to cardiac function. *Clin Endocrinol (Oxf).* 2000;53:61-8.  
501 [39] McDevitt TC, Laflamme MA, Murry CE. Proliferation of cardiomyocytes derived from  
502 human embryonic stem cells is mediated via the IGF/PI 3-kinase/Akt signaling pathway. *J*  
503 *Mol Cell Cardiol.* 2005;39:865-73.  
504 [40] Azar WJ, Azar SH, Higgins S, Hu JF, Hoffman AR, Newgreen DF, et al. IGFBP-2  
505 enhances VEGF gene promoter activity and consequent promotion of angiogenesis by  
506 neuroblastoma cells. *Endocrinology.* 2011;152:3332-42.  
507 [41] Mallick A, Januzzi JL, Jr. Biomarkers in acute heart failure. *Rev Esp Cardiol (Engl Ed).*  
508 2015;68:514-25.

509

510 **FIGURE LEGENDS**

511 **Figure 1:** (A) **Kaplan-Meyer survival curves for the combined Toulouse University**  
512 **Hospital and Atlanta Outpatient HF cohorts.** Significance of stratification according to  
513 the plasma IGFBP2 quartile group. Cardiovascular mortality trend was evaluated with a  
514 Log-rank test. HR: hazard ratio Q: quartile.

515 **(B) Kaplan-Meyer survival curves for the Maastricht University hospital cohort**  
516 **according to the plasma IGFBP2 quartile group.** Significance of stratification of  
517 patients according to IGFBP2 quartile to estimate mortality trend was evaluated with a  
518 Log-rank test. HR: hazard ratio. Q: quartile.

519

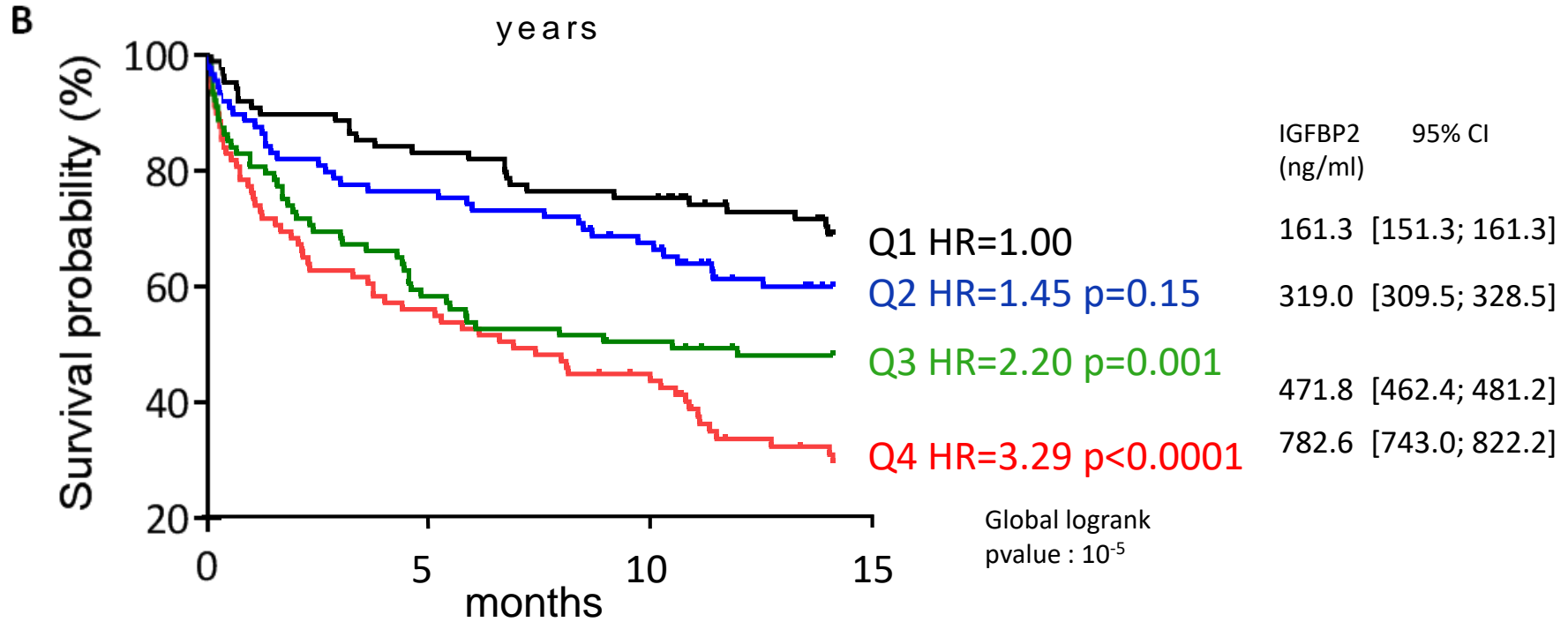
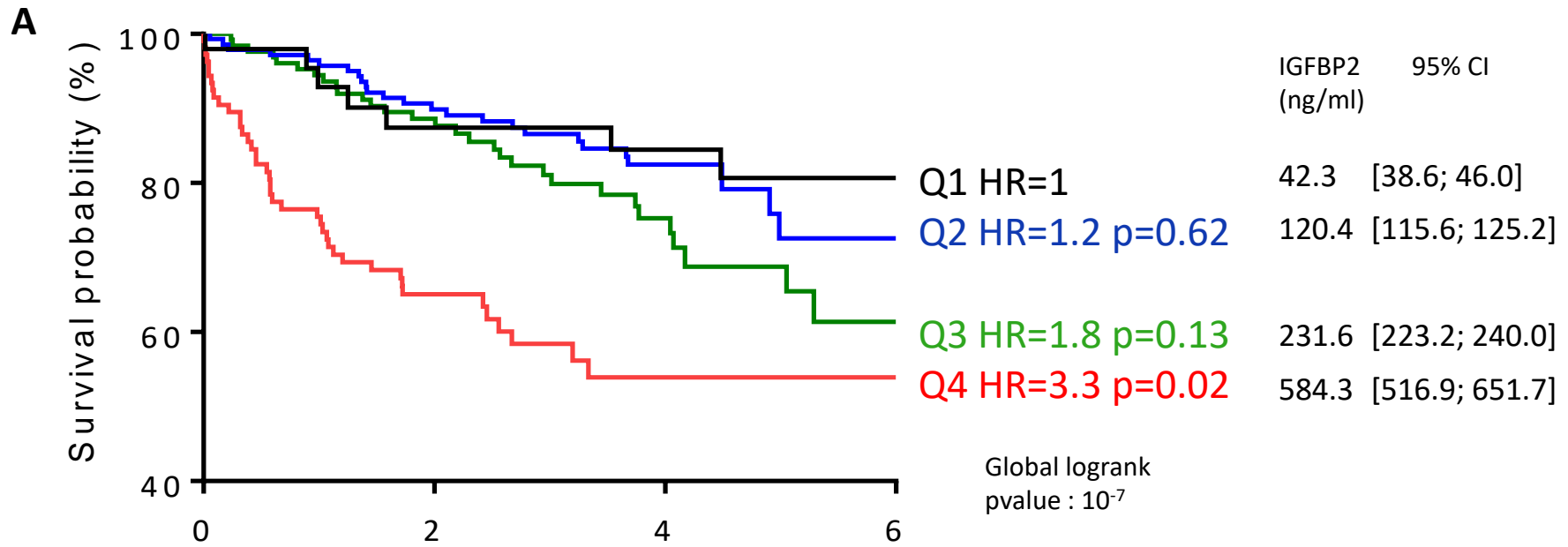
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**FIGURE 1**



**Table 1**

Demographic and clinical characteristics of dataset, stratified by site

Patients characteristics	Site			Comparison of chronic HF patients' values
	Maastricht ED cohort (N=367)	Atlanta Cardiomyopathy Consortium cohort (N=306) †	IBLOMAVED Chronic stable HF cohort (N=197)	
Median Age [IQR], years	78 [71-85] (N=367)	57 [49-65] (N=306)	60 [51-71] (N=192)	<b>P=0.0079</b>
Gender, Male, %	54 (N=198/367)	64 (N=196/306)	79 (N=156/197)	<b>P=0.00044</b>
<b>Medical History, %</b>				
Atrial fibrillation	38 (N=139/365)	15 (N=46/306)	-	
Coronary Artery Disease	50 (N=181/363)	-	-	
COPD	19 (N=68/365)	-	-	
Hypertension	-	-	42 (N=73/175)	
Diabetes	33 (N=114/346)	33 (N=102/306)	24 (N=42/178)	<b>P=0.036</b>
Dyslipidemia	-	-	28 (N=49/176)	
Smoking	-	-	62 (N=109/175)	
<b>HF Etiology, %</b>				
Ischemic	-	41 (N=125/306)	45 (N=80/178)	P=0.39
<b>Medication, %</b>				
ACE inhibitor	37 (N=134/361)	78 (N=239/306)	77 (N=143/186)	P=1
ARAI	22 (N=68/306)	-	14 (N=27/187)	
Beta-blocker	61 (N=220/361)	91 (N=279/306)	71 (N=132/186)	<b>P=1.5.10<sup>-8</sup></b>
Diuretic	63 (N=228/361)	83 (N=254/306)	83 (N=156/187)	P=1
MRA	-	44 (N=135/306)	-	
Antithrombotic agent	78 (N=280/361)	-	52 (N=97/186)	
Hypolipidemic agent	-	-	45 (N=84/187)	
<b>Median [IQR] Admission labs</b>				
BNP, ng/l	-	220 [71-659] (N=240)	289 [114-672] (N=173)	P=0.21
NT-proBNP, ng/l	704 [331-1459] (N=366)	-	-	
Overall IGFBP2 mg/l	393 [240 – 551] (N=367)	172 [76-274] (N=306)	165 [75-391] (N=194)	<b>P=0.00070</b>
Estimated Glomerular Filtration Rate, mL/min/1.73 m <sup>2</sup>	44 [29-60] (N=367)	64 [50-82] (N=286)	60 [46-76] (N=181)	P=0.19
C reactive protein, mg/l	14 [4-30]* (N=336)	-	8.3 [5-24] (N=165)	
Na <sup>+</sup> , mmol/l	139 [136-141] (N=366)	139 [137-140] (N=289)	138 [136-140] (N=172)	<b>P=0.025</b>
ALT, U/ml	29 [20-44] (N=357)	-	25 [20-39]	
<b>Median [IQR] Admission vitals</b>				
SBP, mmHg	138 [115-155] (N=348)	110 [100-124] (N=300)	112 [100-133] (N=112)	P=0.051
DBP, mmHg	75 [64-90] (N=348)	70 [60-80] (N=300)	70 [60-80] (N=112)	P=0.77
Heart rate, bpm	90 [73-112] (N=335)	72 [64-80] (N=301)	80 [70-92] (N=115)	<b>P&lt;0.0001</b>
<b>Echocardiography</b>				
Median [IQR] LVEF, %	38 [25-53] (N=249)	35 [26-50] (N=306)	30 [25-40] (N=186)	<b>P=0.010</b>
LVEF < 40%, %	51 (N=127/249)	60 (N=185/306)	76 (N=142/186)	<b>P=0.00048</b>

\*hs-CRP † Atlanta cardiomyopathy consortium cohort was 51% white, 45% African American and 63% of the 306 patients had ICD/CRT device.

**Table 2**

Combined Toulouse University Hospital and Atlanta Outpatient HF cohorts,

(A) Multivariate Cox proportional hazards regression analysis

	HR* (95% CI)	P	HR† (95% CI)	P
Age, per y	<b>1.02</b> (1.0-1.04)	<b>0.03</b>	<b>1.02</b> (1.0-1.04)	<b>0.02</b>
Gender, male = 1	1.10 (0.7-1.7)	0.67	-	-
Diabetes	1.34 (0.8-2.1)	0.22	-	-
Creatinine per $\mu\text{mol/l}$	0.99 (0.99-1.0)	0.49	-	-
(IGFBP2) <sup>‡</sup> Q4	<b>2.14</b> (1.3-3.5)	<b>0.002</b>	<b>2.04</b> (1.3-3.3)	<b>0.003</b>
(BNP) <sup>‡</sup> Q4	<b>2.05</b> (1.3-3.3)	<b>0.004</b>	<b>2.03</b> (1.3-3.3)	<b>0.004</b>

\* Adjusted model for age, gender, diabetes status, creatinine, BNP and IGFBP2, significance level of overall model fit  $p=0.0002$  with 23% number events for a total number of cases of 503.

† Stepwise method for selection of better predictors (enter variable if  $p<0.1$ , remove variable if  $p>0.2$ ).

‡ HR=1 (referent) cases with concentration lower than the 75<sup>th</sup> percentile i.e. 391 ng/ml and 274 ng/ml for IGFBP2 and 672 pg/ml and 659 pg/ml for BNP, in Toulouse University Hospital and Atlanta Outpatient HF cohorts, respectively. Q: quartile.

(B) Net Reclassification Improvement (NRI) analysis and Integrated Discrimination Improvement (IDI).

Model	NRI (%)	NRI p value	IDI (%)	IDI p value
Model 1* + BNP <sup>‡</sup>	27.6 <sup>§1</sup>	<b>0.04</b>	1.6	0.06
	25.7 <sup>§2</sup>	<b>0.03</b>	1.9	<b>0.03</b>
	27.3 <sup>§3</sup>	<b>0.04</b>	3.0	<b>0.01</b>
Model 1* + IGFBP2 <sup>‡</sup>	40.4 <sup>§1</sup>	<b>0.01</b>	2.9	<b>0.0005</b>
	31.2 <sup>§2</sup>	<b>0.04</b>	3.1	<b>0.0005</b>
	31.5 <sup>§3</sup>	<b>0.02</b>	4.2	<b>0.0005</b>
Model 2 <sup>†</sup> + IGFBP2 <sup>‡</sup>	40.4 <sup>§1</sup>	<b>0.0005</b>	2.9	<b>0</b>
	25.9 <sup>§2</sup>	<b>0.04</b>	3.0	<b>0.01</b>
	28.5 <sup>§3</sup>	<b>0.01</b>	3.9	<b>0.01</b>

\* Model 1 includes the following parameters: age, gender, diabetes status and creatinine ( $\mu\text{mol/l}$ ). † Model 2 includes Model 1 with BNP<sup>‡</sup> level. ‡ Plasmatic values dichotomized as =0 if inferior or =1 if superior or equal to the 75<sup>th</sup> percentile i.e. 659 pg/ml and 672 pg/ml for BNP in Atlanta and Toulouse cohort, respectively, and 274 ng/ml and 391 ng/ml for IGFBP2 in Atlanta and Toulouse cohort, respectively. <sup>§1, §2, §3</sup> follow-up time of 1, 2 or 3 years.

**Table 3****(A)** Maastricht ED cohort, multivariate Cox proportional hazards regression analysis.

	HR* (95% CI)	P	HR† (95% CI)	
Age, per y	1.01 (0.99 - 1.03)	0.152	1.01 (1.00 - 1.04)	0.075
Gender, male = 1	0.97 (0.70 - 1.34)	0.835	-	-
(IGFBP2) <sup>‡</sup> G4	<b>1.64</b> (1.15 - 2.35)	<b>0.006</b>	<b>1.69</b> (1.18 - 2.41)	<b>0.004</b>
(NT-proBNP) <sup>‡</sup> G4	1.31 (0.91 – 1.89)	0.144	-	-
ALT per U/ml	0.99 (0.99 – 1.00)	0.395	-	-
hs-CRP per mg/l	0.99 (0.99 – 1.00)	0.991	-	-
Creatinine per 10 µmol/l	<b>1.02</b> (1.00 – 1.03)	<b>0.006</b>	<b>1.02</b> (1.01 - 1.03)	<b>0.005</b>
Na <sup>+</sup> per mmol/l	1.00 (0.97 – 1.05)	0.755	-	-

\* Adjusted model for age, gender, ALT, hs-CRP, creatinine, NT-proBNP and IGFBP2, significance level of overall model fit  $p < 0.0001$  with 46% number events for a total number of cases of 367.

† Stepwise method for selection of better predictors (enter variable if  $p < 0.1$ , remove variable if  $p > 0.2$ ).

‡ HR=1 (referent) cases with concentration lower than the 75<sup>th</sup> percentile i.e. 551 ng/ml and 1459 pg/ml for IGFBP2 and NT-proBNP, respectively.

**(B)** Maastricht ED cohort, Net Reclassification Improvement (NRI) analysis **and Integrated Discrimination Improvement (IDI).**

Model	NRI (%)	NRI p value	IDI (%)	IDI p value
Model 1* + NT-proBNP (pg/ml)	6.2	0.458	0.3	0.58
Model 1* + IGFBP2 (ng/ml)	<b>18.7</b>	<b>0.03</b>	<b>3.9</b>	<b>0.02</b>
Model 2 <sup>†</sup> + IGFBP2 (ng/ml)	<b>19.1</b>	<b>0.02</b>	<b>3.8</b>	<b>0.05</b>

\* Model 1 includes the following parameters: age, gender, sodium (mmol/L), ALT (U/ml), hs CRP (mg/l) and creatinine (µmol/l).

† Model 2 includes Model 1 and NT-proBNP (pg/ml).

1-year follow-up.