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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^e) 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 β 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

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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.

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FIGURE 1

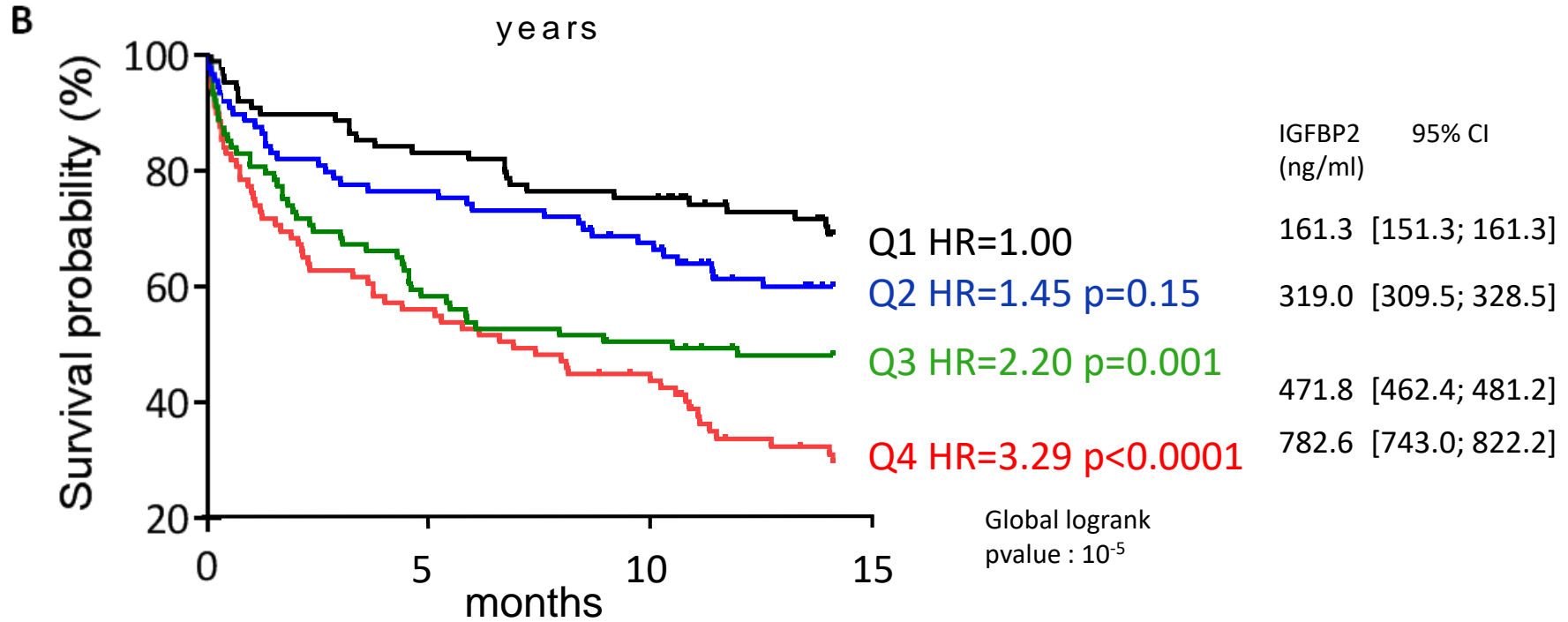
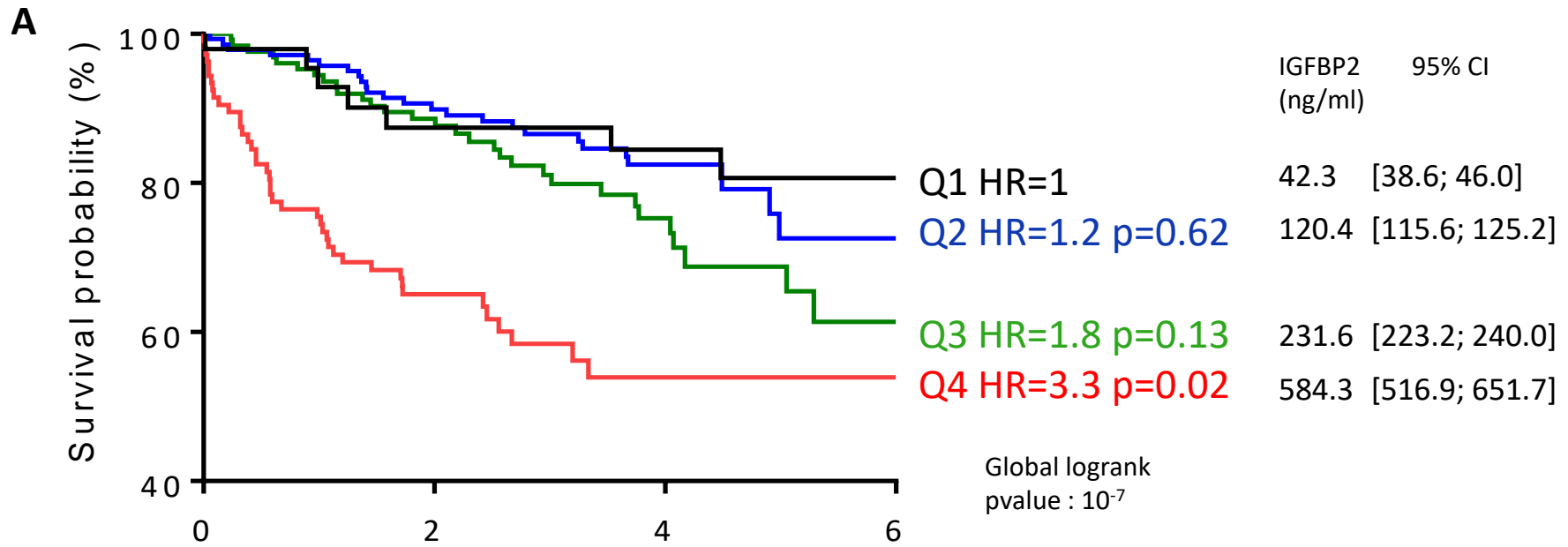


Table 1

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)	P=0.0079
Gender, Male, %	54 (N=198/367)	64 (N=196/306)	79 (N=156/197)	P=0.00044
Medical History, %				
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)	P=0.036
Dyslipidemia	-	-	28 (N=49/176)	
Smoking	-	-	62 (N=109/175)	
HF Etiology, %				
Ischemic	-	41 (N=125/306)	45 (N=80/178)	P=0.39
Medication, %				
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)	P=1.5.10⁻⁸
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)	
Median [IQR] Admission labs				
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)	P=0.00070
Estimated Glomerular Filtration Rate, mL/min/1.73 m ²	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 ⁺ , mmol/l	139 [136-141] (N=366)	139 [137-140] (N=289)	138 [136-140] (N=172)	P=0.025
ALT, U/ml	29 [20-44] (N=357)	-	25 [20-39]	
Median [IQR] Admission vitals				
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)	P<0.0001
Echocardiography				
Median [IQR] LVEF, %	38 [25-53] (N=249)	35 [26-50] (N=306)	30 [25-40] (N=186)	P=0.010
LVEF < 40%, %	51 (N=127/249)	60 (N=185/306)	76 (N=142/186)	P=0.00048

*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	1.02 (1.0-1.04)	0.03	1.02 (1.0-1.04)	0.02
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) [‡] Q4	2.14 (1.3-3.5)	0.002	2.04 (1.3-3.3)	0.003
(BNP) [‡] Q4	2.05 (1.3-3.3)	0.004	2.03 (1.3-3.3)	0.004

* 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 75th 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 [‡]	27.6 ^{§1}	0.04	1.6	0.06
	25.7 ^{§2}	0.03	1.9	0.03
	27.3 ^{§3}	0.04	3.0	0.01
Model 1* + IGFBP2 [‡]	40.4 ^{§1}	0.01	2.9	0.0005
	31.2 ^{§2}	0.04	3.1	0.0005
	31.5 ^{§3}	0.02	4.2	0.0005
Model 2 [†] + IGFBP2 [‡]	40.4 ^{§1}	0.0005	2.9	0
	25.9 ^{§2}	0.04	3.0	0.01
	28.5 ^{§3}	0.01	3.9	0.01

* Model 1 includes the following parameters: age, gender, diabetes status and creatinine ($\mu\text{mol/l}$). † Model 2 includes Model 1 with BNP[‡] level. ‡ Plasmatic values dichotomized as =0 if inferior or =1 if superior or equal to the 75th 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. ^{§1, §2, §3} 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) [‡] G4	1.64 (1.15 - 2.35)	0.006	1.69 (1.18 - 2.41)	0.004
(NT-proBNP) [‡] 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	1.02 (1.00 – 1.03)	0.006	1.02 (1.01 - 1.03)	0.005
Na ⁺ 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 75th 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)	18.7	0.03	3.9	0.02
Model 2 [†] + IGFBP2 (ng/ml)	19.1	0.02	3.8	0.05

* 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.