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Early View

Original article

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Fibroblast Growth Factor-21 Predicts Outcome in Community-Acquired Pneumonia

Secondary Analysis of two Randomized Controlled Trials

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This article has an online data supplement, which is accessible from this issue's table of content online.

Take Home Message:
Fibroblast growth factor-21 predicts higher risk for clinical instability and 30-day mortality in moderate-to-severe community-acquired pneumonia.

Abstract

Acute systemic inflammatory conditions come along with profound alterations of metabolism. However the role of FGF21, a recently identified central regulator of metabolism is largely unknown in community-acquired pneumonia.

This study aims to characterize the pattern of FGF21 in pneumonia and associations with disease severity and outcome.

This is a secondary analysis of two independent multicenter randomized controlled trials in patients presenting to the emergency department with community-acquired pneumonia. Primary and secondary efficacy parameters included 30-day mortality, length of hospital stay, time to clinical stability and duration of antibiotic treatment.

A total of 509 patients were included in the analysis. FGF21 levels at admission strongly correlated to disease severity, as measured by pneumonia severity index. Increased levels of FGF21 were associated with prolonged time to clinical stability, antibiotic treatment and hospitalization. FGF21 levels at admission were significantly higher in non-survivors than in survivors, yielding a 1.61-fold increased adjusted odds ratio of 30-day mortality (95% CI, 1.21−2.14; p=0.001). Moreover, FGF21 was found to identify patients for 30-day mortality with superior discriminative power compared to than routine diagnostic markers.

In moderate-to-severe CAP patient with higher levels of FGF21 were at increased risk for clinical instability, prolonged hospitalization and 30-day all-cause mortality.

Keywords: Pneumonia, Metabolism, Time to clinical stability, Mortality, Corticosteroids, Outcome prediction.

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Introduction

Community-acquired pneumonia (CAP) is a major cause of morbidity and mortality and related socioeconomic costs worldwide [1, 2]. CAP-related mortality remains high despite recent advances in medical care. The World Health Organization (WHO) estimated in 2016 that lower respiratory tract infections were the fourth leading cause of death globally and the number one cause of death in low-income countries [3]. Clinical signs and symptoms such as fever and chills or routine laboratory parameters such as c-reactive protein or white blood cell count have shown to be of only limited value for the management and prognosis of severe lower respiratory tract infections [4, 5]. Hence, prognostic scoring rules have been developed with the aim to integrate the complex pathophysiological changes in order to predict severity and outcome of CAP and to guide therapy. Of these the pneumonia severity index (PSI) and the CURB-65 are the most common prognostic classification scores [6]. However, calculating these scores has shown to be reasonably complex and time-consuming, which leads to the fact that they are often not used and that the majority of clinicians are not able to accurately calculate the scores [7]. Against this background a simple and reliable approach is needed to help identify patients at risk and streamline management decisions in patients presenting with severe CAP.

Fibroblast growth factor-21 (FGF21) has recently been identified as a central regulator of metabolism via adaptation of glucose homeostasis, ketogenesis, insulin sensitivity, and lipid metabolism [8, 9]. Furthermore, it probably plays a major role in the regulation of energy expenditure, enhancing substrate utilization and potently inducing thermogenesis in brown adipose tissue (BAT) [10–13].

Several studies have suggested the potential role of FGF21 in the maintenance and regulation of energy homeostasis and mitochondrial function in conditions of metabolic stress [14, 15]. The capacity of FGF21 to facilitate thermogenesis in BAT suggests that it might as well be involved in the elevation of body temperature in systemic inflammatory states.

However, while acute systemic inflammatory conditions come along with profound alterations of metabolism such as hyperglycemia, insulin resistance, and mitochondrial dysfunction [16–18], the role of the central regulator FGF21 is still unknown. Emerging data suggest that FGF21 is a positive acute phase protein since systemic inflammation has been shown to induce an approximately 2-fold increase in serum FGF21 which might contribute to adipose tissue lipolysis, and ketone production that occur during inflammation [19, 20]. In addition, studies have shown that FGF21 potentially plays an anti-inflammatory and immunoregulatory role by downregulation of the pro-inflammatory cytokine Interleukin (IL)-1β and up-regulation of the anti-inflammatory cytokine IL-10 [20]. FGF21 might therefore protect from overwhelming systemic inflammation in severe inflammatory states such as lower respiratory tract infections.

In this study we investigated the potential role of FGF21 as predictive biomarker in CAP, using samples and clinical data from two large, well-defined patient cohorts with moderate-to-severe lower respiratory tract infections (ProCAP trial (SRCTN04176397) [21], and STEP trial (NCT00973154) [22]). The main aims of this study were to assess and confirm the clinical use of FGF21 for early diagnosis and clinical guidance of therapy, to predict adverse outcome and to evaluate its interaction with adjunct corticosteroid treatment in CAP.

Materials and Methods

Study subjects and design This is a secondary analysis of two investigator-initiated, parallel-group, randomized, multicenter trials involving patients with all severities of CAP admitted to the emergency department. Full details of the trial designs and results have been published [21–23]. In brief, consecutive patients (18 years or older) presenting with CAP were enrolled at emergency departments or medical wards of tertiary care hospitals in Switzerland within 24 h of presentation.

Patients in the Procalcitonin-guided Reduction of the Duration of Antibiotic Therapy in Community-acquired Pneumonia trial (ProCAP) were randomized (1:1 ratio) to either procalcitonin-guided antibiotic treatment or usual care [21]. In the Corticosteroid treatment for community-acquired pneumonia trial (STEP), patients were randomly assigned (1:1 ratio) to receive either 50 mg of prednisone or placebo daily for 7 days as adjunct treatment. Patients, treating physicians, investigators, and data assessors were masked to treatment allocation [22].

The conduct of the trials adhered to the declaration of Helsinki and Good Clinical Practice Guidelines, and ethical committees of all participating hospitals approved the studies before patient recruitment. The trials were registered on www.isrctn.com and ClinicalTrials.gov, respectively (ProCAP trial study-ID: SRCTN04176397; STEP trial study-ID: NCT00973154).

Methods Informed consent was obtained within 24 hours of admission to hospital. All patients were treated according to international CAP consensus guidelines [24]. Baseline data included medical history, relevant comorbidities, clinical variables

relating to pneumonia and all variables required for the calculation of the pneumonia severity index (PSI) [25]. Clinical study data were systematically gathered up to obtain 30-day outcome after admission.

Main outcome parameters included time to effective hospital discharge (LOS), 30-day all-cause mortality, duration of intravenous and overall antibiotic treatment, time to clinical stability (TTCS, defined as the time to stabilization of vital signs at two consecutive measurements ≥ 12 h apart [22]), admission to intensive-care-unit (ICU), and CAP complications (including recurrence, acute respiratory distress syndrome, empyema, nosocomial infections until day 30, severe adverse events possibly related to CAP, ICU admission and re-admission to hospital).

Analysis of fibroblast growth factor 21 (FGF21) Blood samples from each patient were collected upon emergency department admission and on day 3 and were subsequently stored at -80°C. For this analysis, remaining serum samples from the ProCAP trial, in total 150 patients were still available. In the STEP trial all patients from the main study site, University Hospital of Basel (n=359, 177 patients allocated to placebo and 182 patients to prednisone, respectively), were included. FGF21 was measured in serum samples using the human FGF21 Quantikine immunoassay (R&D systems, Minneapolis, MN) for the ProCAP samples and the human FGF21 Ella Simple Plex assay (ProteinSimple, San Jose, California, USA) for STEP samples, respectively. Both assays are based on an identical monoclonal antibody specific for human FGF21, which detects total FGF21 independent of plasmatic proteolytic on N- or C-terminal cleavage [26]. The two assays correlate well (R^2=0.97, information provided by R&D / Protein Simple). The Quantikine ELISA has a limit of detection for human FGF21 of 8.69 pg/mL

and an inter-assay coefficient of variation (CV) of 5.2 to 10.9%. The Simple Plex assay has a limit of detection of 3.74 pg/mL and an inter-assay CV of 7.8%; mean values of three independent measurements were taken for analysis. Reference values for young healthy subjects were measured in 56 participants from the FluvaBAT and MIBAT studies (clinicaltrials.gov IDs NCT03189511 and NCT02682706, respectively). Reference values for elderly healthy subjects were measured in serum samples from 40 patients from an endocrine outpatient clinic in Basel, Switzerland. All healthy participants gave written informed consent.

Statistical analysis Unless stated otherwise, categorical variables are expressed as n (percentage) and continuous variables as medians (interquartile range [IQR]). The distribution of FGF21 was right-skewed. After logarithmic transformation with a base of 10, the distribution approximated a normal distribution.

For analyses of associations of comorbidities with baseline levels of FGF21 univariate and multivariate regression models were used. Unadjusted and adjusted estimates of the effect size and corresponding 95% confidence intervals (CIs) using linear, logistic, or Cox proportional hazards regression as appropriate. All multivariate models were adjusted for the same variables: patient age, gender, diabetes mellitus, congestive heart failure (CHF), and renal insufficiency. Analyses on STEP study participants were additionally adjusted for randomized treatment. Kaplan–Meier curves were used to illustrate TTCS and LOS based on FGF21 tertiles (highest versus lower two). Log-rank tests were used to detect differences between groups. Receiver operating characteristic (ROC) analyses were performed to analyze the discriminative power to identify patients at risk for 30-day mortality. Correlation analyses were performed calculating Spearman-Rho (r). For

analyses, consistent clinical and laboratory variables of the ProCAP and STEP trials were pooled. All statistical analyses were performed using STATA 14.2 (Stata Corp, College Station, TX, USA) and tests were done at a two-sided 5% significance level with two-sided 95% CIs.

Results

Patient characteristics

Overall, 509 patients treated at the University Hospital Basel were included in this study, 150 from the ProCAP and 359 from the STEP trial population. Baseline characteristics of included patients are presented in **Table 1** and **Table 2**, respectively (a combined table of baseline characteristics is included in the Online Supplemental Material **Table S1**). Patients were at median 73 years old and 62.0% were male in ProCAP and 75 years old with 63.2% male in STEP. The burden of pre-existing pulmonary disease was relatively low in both studies with a history of chronic obstructive pulmonary disease (COPD) in 21.3% and 18.9%, respectively. from the prevalence of diabetes mellitus type 2 was 23.3% and 22.8%, respectively. Significantly fewer patients had pre-existing congestive heart failure (CHF) in the ProCAP trial (6.0%) in the STEP trial (20.6%). Overall, more than half of the patients (57.3% and 54.6%) had severe pneumonia classified in the high-risk PSI classes IV and V, respectively.

Association of FGF21 with pneumonia severity

At baseline, serum FGF21 levels were highly increased (456.5 pg/mL; interquartile range (IQR) 181.2−1127.9) compared with values in a population of elderly healthy controls (140.2 pg/mL; IQR 81.1−161.8; $p<0.001$) and almost ten-fold higher than

in young healthy controls (50.4 pg/mL; IQR 13.7−113.3; p<0.001). They subsided until day 3 when they were still 2-fold elevated (299.9 pg/mL; IQR 157.9−659.9) when compared to elderly healthy controls (characteristics of healthy controls are shown in Online Supplemental Material **Table S2a** and **S2b**). We found a significant positive correlation of FGF21 levels at emergency admission with procalcitonin (PCT) levels (r=0.186, R^2= 0.045; *p*<0.001), but not with c-reactive protein (CRP) levels (r=-0.058, R^2= 0.001; *p*=0.19) (**Figure S3** and **S4**). There was likewise no correlation of FGF21 levels with body temperature (in-ear) at emergency admission (**Figure S5**).

On admission, levels of FGF21 increased step-wise with higher PSI-classes of CAP severity (**Figure 1A and 1C** for each study separately and **Figure S1** for pooled cohort). There was a linear correlation of log-transformed FGF21 with the PSI-score both in ProCAP (R^2=0.1667, p<0.001) as well as in STEP cohort (R^2=0.1604, p<0.001) (see Online Supplemental Material, **Figure S6**). Patients with severe pneumonia categorized into PSI-classes IV or V had markedly higher baseline levels of FGF21 (717.3 pg/mL; IQR 291.5−1530.1) than patients with mild to moderate severity categorized to PSI-classes I, II or III (270.5 pg/mL; IQR 123.3−626.9); *p*<0.001 (**Figure S2**). In ROC analyses, FGF21 significantly better discriminated patients with high-risk (PSI IV-V) from those with low-risk (PSI I-III) when compared to established biomarkers. The area under the curve (AUC) for FGF21 was 0.68 (0.64−0.73); for CRP 0.48 (95% CI, 0.43−0.53; p<0.001), for PCT 0.56 (95% CI, 0.51−0.62; p=0.02) and for white blood cell count (WBC) 0.46 (95%CI 0.41−0.51; p<0.001) (**Table S4**).

Patients in the highest tertile of FGF21 levels (>833.3 pg/mL) required 2 days longer to achieve clinical stability than patients in the lower two tertiles (6.0 days; IQR 3.0−10.0 vs. 4.0 days; IQR 2.0−7.4). The adjusted hazard ratio (HR) for Cox regression of FGF21 at baseline was 0.87 (95% CI, 0.80−0.95; p=0.002); a HR < 1.0 corresponding to prolonged time to clinical stability (**Figure 2A** and **Table 3**; data shown for each study separately in **Table S5** and **Table S6**). Accordingly, higher FGF21 was associated with longer duration of intravenous and total antibiotic treatment for each step-wise increase in logFGF21 (**Table 3**).

FGF21 and clinical outcome

Median length-of-hospital stay was significantly longer in patients in the highest FGF21 tertile (10.0 days; IQR 7.0–16.0) compared to the lower two tertiles of FGF21 levels (8.0 days; IQR 5.0–14.0), resulting in an adjusted hazard ratio (HR) of 0.93 (95% CI, 0.87–0.99, p=0.03; **Figure 2B** and **Table 3**; data shown for each study separately in **Table S5** and **Table S6**). Overall, complications associated with CAP (i.e., acute respiratory distress syndrome, empyema, respiratory failure with intubation, persistence of pneumonia, and CAP-associated mortality) tended to be higher in the 3rd tertile of FGF21 compared to the lower two tertiles (44.5% vs. 30.4%); however the adjusted odds ratio (OR) did not meet statistical significance (p=0.15). The rate of ICU admissions was increased (13.6% in 3rd tertile vs. 8.5% in first two tertiles). For each increment in baseline logFGF21 levels the adjusted OR was 1.32 (95% CI, 1.07–1.65, p=0.01;**Table 3**).

FGF21 and CAP-associated mortality

Levels of FGF21 were significantly higher in non-survivors than in corresponding survivors (1307.6 pg/mL vs. 416.7 pg/mL; *p*<0.001; **Figure 1B**). In fully adjusted logistic regression analyses, admission levels of FGF21 were associated with an adjusted OR of 1.61 for 30-day mortality for each step-wise increase in logFGF21 (95% CI, 1.21−2.14; *p*=0.001) (**Table 3**).

When compared to PCT and CRP, FGF21 was superior for the identification of patients at risk for 30-day mortality as assessed by comparisons of AUC of the related ROC curves: baseline FGF21 levels had an AUC of 0.73 (0.65-0.82) which was significantly superior than CRP (AUC 0.47; (0.37-0.58); p<0.001), PCT (AUC 0.62 (0.53-0.71); p=0.03), and WBC (AUC 0.31 (0.22-0.39); p<0.001), respectively (**Figure 3** and **Table 4**).

When compared to prognostic scoring scales, baseline FGF21 levels performed better than the CURB65 score and comparable to the PSI score (**Table 4** and **Figure 3**). Accordingly, of the 32 patients who died within 30 days only two patients had an admission FGF21 level below the 95th percentile derived from the elderly healthy reference population. Both patients were not admitted to ICU and had a PSI score of 93 and 94 points which was just above the lower limit of 91 points to be classified into PSI class IV. In comparison, of the 32 patients who died, one patient was misclassified by the PSI into the low-to-intermediate risk classes I-III. This patient had a baseline FGF21 of 369.7 pg/mL and would have been correctly categorized as high-risk by the admission FGF21 level.

When clinical outcomes were analyzed for each trial population separately, the association with mortality was still distinctive despite reduced statistical power (**Table S5** and **Table S6**).

Associations of baseline FGF21 levels with demographic characteristics, comorbidities and clinical variables

Multivariate linear regression models were used to investigate predictors of increased levels of FGF21 at emergency admission. In univariate analysis, age was associated with increased levels of FGF21, however after multivariable adjustment this association was no longer significant (*p*=0.59) (**Table 5**). While patients with a history of congestive heart failure (CHF) (adjusted difference 226.9 pg/mL; 95% CI, 13.7−440.1; *p*=0.04) and with chronic kidney disease (CKD) (adjusted difference 255.2 pg/mL; 95% CI, 72.0−438.4; *p*=0.006) had significantly higher levels of FGF21 at baseline, male gender was associated with lower levels (adjusted difference -238.4 pg/mL; 95% CI, -396.4−(-80.3); *p*=0.003) (**Table 5**).

Effects of corticosteroid treatment on FGF21

Patients in the STEP trial were randomly allocated to receive a treatment with either prednisone 50 mg daily or placebo for 7 days. Prednisone treatment was associated with an accelerated decline in FGF21 levels from 592.8 pg/mL (IQR 231.5−1336.0) to 250 pg/mL at day 3 (IQR 134.6−489.5), as compared to almost no change in the placebo group. Overall, there was a significant between-group difference of -811.3 pg/mL (95% CI, -1448.2−(-174.2); *p*=0.01) (**Figure S7**).

Temporal dynamics of FGF21 and mortality

We calculated the change in FGF21 levels from day 1 to day 3 (deltaFGF21) in the patient cohort from the STEP trial. Non-surviving patients were prone to have an increase in FGF21 levels when compared to survivors (p=0.06, **Figure 4A**). When considering only patients randomized to placebo, deltaFGF21 was significantly higher in the patients who died, while it remained stable in survivors (p=0.002, **Figure 4C**).

Discussion

The key findings of this study involving patients with moderate-to-severe CAP from two randomized-controlled trials are threefold: First, serum levels of FGF21 measured on emergency presentation were markedly increased in the context of systemic inflammation, when compared to healthy volunteers and reference populations [10, 27]. Second, FGF21 significantly correlated with severity and outcome of CAP, yielding a prognostic accuracy as high as the PSI and higher than inflammatory parameters commonly measured in clinical practice. Third, adjunct treatment with corticosteroids, which potently improved hazards for achieving clinical stability, led to significant declines of FGF21 as compared to placebo.

Profound elevations of FGF21 at admission suggest that it might play a role in acute metabolic adaptations in the context of systemic inflammatory responses. In fact, compared to data from healthy volunteers, levels were manifold increased and remained elevated even after three days [8]. Interestingly, patients with CHF and CKD were found to have more pronounced increases in FGF21. This is in line

with previous data showing that levels of FGF21 are progressively increased from early to end stages of CKD [28, 29].

So far, FGF21 has been considered being a starvation-induced metabolic hormone inducing genes implied in ketogenesis and beta-oxidation in mice [30]. However, in humans its role is less well characterized: It has been shown to increase only after prolonged fasting of at least 7 days duration [31], making it unlikely to facilitate acute physiologic adaptations to ketogenesis and beta-oxidation in humans. Additionally, it has been demonstrated that FGF21 is increased in response insulin, such as after an oral glucose tolerance test [32] or in states of insulin resistance [33]. Though, when compared to chronic metabolic conditions, levels of FGF21 were considerably higher in the majority of included CAP patients. In fact, recent studies have demonstrated that FGF21 was notably elevated in states of systemic inflammation such as systemic inflammatory response syndrome (SIRS) and sepsis, both in rodents [19] and humans, respectively [27, 34]. Our results, describe for the first time an association of FGF21 with disease severity and outcome in CAP. FGF21 significantly correlated with disease severity (i.e. pneumonia severity index) and was strongly associated with prolonged time to clinical stability, antibiotic treatment and length of hospital stay. In line with a role for FGF21 in sepsis, its levels correlated relatively well with PCT, which is a well-established biomarker for bacterial infection and sepsis [35]. This notion is supported by animal data demonstrating release of FGF21 into the circulation in response to administration of bacterial lipopolysaccharides. Due to limited data on the causative pathogens of pneumonia in the STEP and Pro-CAP

trials we could not establish whether FGF21 is especially elevated in bacterial versus viral infections of the lower respiratory tract system.

Moreover, it seems unlikely that the rise in FGF21 in our patient cohorts was due to reduced food intake in response to acute illness. It has been suggested that the rise of FGF21 in critically ill patients resulted from a strongly up-regulated hepatic FGF21 expression (as observed in animal models), possibly driven at least in part by mitochondrial damage [27]. Indeed, FGF21 has been demonstrated to be a sensitive biomarker of mitochondrial dysfunction [36]. Another factor to be taken into account is the relative insulin resistance caused by acute illness. Indeed, a further analysis of the FGF21 levels in the STEP trial showed a weak albeit statistically significant association of FGF21 levels with plasma glucose levels on admission, R^2=0.025, p<0.001 (supplemental **Figure S8**). However, given the moderate degree of chronic FGF21 elevation seen in diabetic patients which are merely about two-fold higher than in healthy subjects [33], this does not fully explain the much higher values seen in CAP patients.

Remarkably, we found that upon admission to the emergency department, patients with FGF21 levels in the highest tertile were at more than 3-fold increased risk of 30-day mortality, indicating that FGF21 could serve as a novel biomarker for outcome prediction. FGF21 was found to perform better than established routine inflammatory markers (i.e. CRP, PCT) for the identification of patients at risk for fatal outcome. Even after multivariable adjustment FGF21 levels were still independently predictive of adverse clinical outcome. FGF21 levels were also compared to commonly used clinical scoring systems.

According to our data, the simple measurement of FGF21 provided prognostic information equivalent to the complex 20-variable pneumonia severity index [37]. However, the PSI clearly performed very good in terms of mortality prediction: Only one patient who was misclassified into the low-risk categories I to III died within 30 days. Nevertheless, a major limitation for the routine use of the PSI score is its laborious calculation. In a study validating the predictive potential of various indices in 731 patients with CAP, the PSI score could be calculated in only 70% of all patients [38], restricting its widespread adoption. Moreover, data from Australia have shown that the PSI is routinely used only in between 6% and 27%. Even emergency and respiratory physicians only infrequently used the PSI and were unable to apply it accurately [7]. We show that FGF21 performed similarly to PSI in predicting 30 day mortality using ROC analysis. Only two of the non-survivors had an admission FGF21 level which was not above the 95th percentile of the healthy elderly reference population. Hence, measurement of FGF21, ideally at the point of care, with a rapid immunoassay could therefore facilitate the fast and correct triage of patients with CAP. Additionally, beside the prognostic value of admission levels of FGF21, our data show that the temporal dynamics of FGF21 during the first three days of CAP were of predictive value, since rising levels were associated with adverse outcome. Against this background, repeated measurements of FGF21 could possibly further facilitate appropriate patient management. However, given the relatively low mortality in our patient populations larger cohorts will be needed to determine whether FGF21 is superior to PSI in determining 30 day mortality or vice versa.

Since experimental data suggest that treatment with exogenous FGF21 may be protective in endotoxemia reducing the mortality, the observed increase in circulating FGF21 during systemic inflammation might even be a protective counterregulatory response [19]. Thus, in addition to the diagnostic value of FGF21, it might as well be a potential treatment option in severe pneumonia with sepsis. Clinical trials in humans are needed to investigate possible therapeutic applications of FGF21 in critical illness.

In animal studies, glucocorticoids have been shown to induce FGF21 in a feed-forward loop: In mice dexamethasone induced FGF21 expression in the liver by acting on the glucocorticoid receptor (GR). The transcription of FGF21 was co-stimulated by the peroxisome proliferator-activated receptor-α (PPARα). FGF21 in turn enhanced gene expression in the adrenal gland via the central nervous system, which resulted in an increase of corticosterone [39]. Since patients from the STEP trial were randomized to a treatment with 50 mg of prednisone per day vs. placebo, we investigated the effects of the corticosteroid treatment on FGF21 levels after three days. In contrast to the animal data, adjunct treatment with prednisone, which was associated with a shorter time to clinical stability, reduced FGF21 levels as compared to placebo. As patients on immunosuppressive medication were by protocol excluded from the STEP trial, the patient's medication as a possible systemic confounder that could have had an immunomodulatory effect, seems unlikely. However, one patient who was incorrectly classified as low-risk by FGF21 levels from the ProCAP trial was on glucocorticoids. This might in fact have reduced FGF21 levels and is a potential caveat when considering the use of FGF21 as a prognostic marker.

Our study has the following limitations: First, the results apply only to patients with more severe pneumonia, who require admission to emergency department and in most cases hospitalization. Second, we performed a secondary analysis of two randomized controlled trials which were not originally designed to explore the current hypothesis. Third, the outcome of pneumonia was significantly better in the more recent STEP trial than in the ProCAP trial. This seems to be primarily due to advances in the management of severe pneumonia over the past years. The main strengths of our study include (i) the validation of the findings in two independent large randomized-controlled trials with well-defined patient cohorts, (ii) the assessment of clinically relevant endpoints, and (iii) the use of robust and precise measurement methods.

In conclusion, in two independent large randomized-controlled trials involving patients with community-acquired pneumonia, FGF21 strongly correlated with disease severity and was identified as an early predictor of adverse outcome. Further studies are required to characterize the role of FGF21 in the regulation of immunometabolism during in the progression and remission of septic conditions and to elucidate whether targeting this pathway would be of therapeutic benefit.

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Author contributions CW, FE, MCC, MJB were involved in the conception, hypothesis delineation, design of the study and interpretation of data. FE and MJB performed the measurements of FGF21. BM, CAB, PS, CM, and MK contributed data and were involved in revising the manuscript critically. All authors approved the final version to be published. FE and MJB had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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Tables

Table 1 - Baseline characteristics and clinical variables of enrolled patients – ProCAP trial

Characteristic/variable	Total cohort (n=150)
General characteristics	
Age, years	73.0 (56.0, 82.0)
Male sex	93 (62.0%)
BMI, kg/m^2	24.8 (22.2, 28.7)
Smoking status	32 (21.3%)
Packyears, years	30.0 (20.0, 50.0)
Comorbidities	
Diabetes mellitus	35 (23.3%)
COPD	32 (21.3%)
Asthma	3 (2.0%)
Heart failure	9 (6.0%)
Coronary artery disease	47 (31.3%)
Cerebrovascular disease	9 (6.0%)
Renal insufficiency	34 (22.7%)
Neoplastic disease	23 (15.3%)
Antibiotic pre-treatment	31 (20.7%)
Clinical variables	
Systolic blood pressure, mmHg	130.0 (113.0, 142.0)
Heart rate, bpm	96.0 (84.0, 108.0)
Respiratory rate, breaths/min	22.0 (18.0, 27.0)
Body temperature, °C [in-ear]	38.5 (37.7, 39.3)
SIRS, points.	3 (2, 4)
PSI class†	
I, II and III	64 (42.7%)
IV and V	86 (57.3%)
PSI, points†	95 (71, 115)
Laboratory values	
C-reactive protein, mg/L	135.5 (74.0, 216.4)
Procalcitonin, ng/dL	0.4 (0.2, 1.4)
White blood cell count, G/L	12.8 (9.0, 15.4)
Fasting glucose, mmol/L	6.8 (5.8, 8.7)

Data are presented as median (IQR) for continuous or n (%) for categorical variables, unless otherwise stated. BMI, body mass index; COPD, chronic obstructive pulmonary disease; SIRS, systemic inflammatory response syndrome; PSI, pneumonia severity

index. †The PSI is a clinical prediction rule to calculate the probability of morbidity and mortality in patients with community-acquired pneumonia [25]; PSI risk class I corresponds to age ≤50 years, and no risk factors (≤50 points), risk class II to <70 points, risk class III to 71–90 points, risk class IV to 91–130 points, and risk class V to >130 points.

Table 2 - Baseline characteristics and clinical variables of enrolled patients – STEP trial

Characteristic/variable	Total (n=359)	Prednisone (n=182)	Placebo (n=177)	*p*-value
General characteristics				
Age, years	75.0 (62.0, 83.0)	77.0 (63.0, 84.0)	74.0 (61.0, 83.0)	0.17
Male sex	227 (63.2%)	115 (63.2%)	112 (63.3%)	0.99
BMI, kg/m^2	26.1 (23.0, 29.4)	25.6 (22.7, 28.7)	26.6 (23.7, 30.2)	0.009
Smoking status	86 (24.0%)	47 (25.8%)	39 (22.0%)	0.40
Packyears, years	10.0 (0.0, 40.0)	10.0 (0.0, 40.0)	10.0 (0.0, 40.0)	0.85
Comorbidities				
Diabetes mellitus	82 (22.8%)	39 (21.4%)	43 (24.3%)	0.52
Insulin treatment	31 (8.6%)	16 (8.8%)	15 (8.5%)	0.91
COPD	68 (18.9%)	38 (20.9%)	30 (16.9%)	0.34
Asthma	18 (5.0%)	7 (3.8%)	11 (6.2%)	0.30
Heart failure	74 (20.6%)	39 (21.4%)	35 (19.8%)	0.70
Hypertension	202 (56.3%)	106 (58.2%)	96 (54.2%)	0.44
Cerebrovascular disease	35 (9.7%)	19 (10.4%)	16 (9.0%)	0.65
Peripheral artery occlusive disease	21 (5.8%)	10 (5.5%)	11 (6.2%)	0.77
Renal insufficiency	131 (36.5%)	69 (37.9%)	62 (35.0%)	0.57
Neoplastic disease	18 (5.0%)	7 (3.8%)	11 (6.2%)	0.30
Antibiotic pre-treatment	78 (21.7%)	39 (21.4%)	39 (22.0%)	0.89
Clinical variables				
Days with symptoms, days	4.0 (2.0, 7.0)	4.0 (2.0, 7.0)	4.0 (2.0, 7.0)	0.68
Systolic blood pressure, mmHg	124.0 (110.0, 140.0)	124.0 (110.0, 139.0)	122.0 (109.0, 141.0)	0.78
Diastolic blood pressure, mmHg	65.5 (58.0, 75.0)	65.0 (57.0, 75.0)	66.0 (58.0, 75.0)	0.77
Heart rate, bpm	85.0 (74.0, 98.0)	85.0 (76.0, 97.0)	87.0 (71.0, 99.0)	0.69
Respiratory rate, breaths/min	20.0 (18.0, 24.0)	20.0 (18.0, 24.0)	20.0 (17.0, 24.0)	0.55
Body temperature, °C [in-ear]	38.0 (37.4, 38.6)	38.0 (37.5, 38.6)	38.0 (37.4, 38.5)	0.65
SIRS, points.	2 (2, 3)	2 (2, 3)	2 (2, 3)	0,94
PSI class[†]				
I, II and III	163 (45.4%)	81 (44.5%)	82 (46.3%)	0.73
IV and V	196 (54.6%)	101 (55.5%)	95 (53.7%)	0.73
PSI, points	95.0 (71.0, 118.0)	98.0 (73.0, 119.0)	93.0 (69.0, 118.0)	0.37
Laboratory values				
C-reactive protein, mg/L	153.6 (80.1, 240.0)	153.9 (80.0, 245.5)	151.0 (81.0, 229.0)	0.85
Procalcitonin, ng/dL	0.4 (0.2, 2.5)	0.5 (0.2, 2.6)	0.4 (0.2, 2.2)	0.45
White blood cell count, G/L	12.0 (8.7, 15.4)	12.5 (9.1, 15.9)	11.6 (7.7, 14.8)	0.064
Fasting glucose, mmol/L	6.6 (5.7, 7.8)	6.4 (5.6, 7.6)	6.7 (5.8, 8.0)	0.41

Data are presented as median (IQR) for continuous or n (%) for categorical variables,

unless otherwise stated. BMI, body mass index; COPD, chronic obstructive pulmonary disease; SIRS, systemic inflammatory response syndrome; PSI, pneumonia severity index.

Table 3 - Overview of FGF21 and relevant clinical outcomes

			Regression Analysis of Baseline FGF21			
	Tertiles 1-2 n=340	Tertile 3 n=169	Univariate regression analysis, OR, HR, coefficient (95% CI)	*p* value	Multivariable adjusted regression analysis, OR, HR, coefficient (95% CI)	*p* value
Death (30-days)	11 (3.2%)	21 (12.4%)	**1.60 (1.25, 2.04)**[b]	**<0.001**	**1.61 (1.21, 2.14)**[b]	**0.001**
Time to effective hospital discharge, days	8.0 (5.0-14.0)	10.0 (7.0-16.0)	**0.89 (0.84, 0.96)**[a]	**0.001**	**0.93 (0.87, 0.99)**[a]	**0.03**
Total duration of antibiotic treatment, days	10.0 (7.0-13.0)	10.0 (7.0-14.0)	**0.68 (0.29, 1.08)**[c]	**0.001**	**0.56 (0.12, 0.99)**[c]	**0.01**
Intravenous antibiotic treatment, days	5.0 (3.0-7.0)	6.0 (5.0-8.0)	**0.91 (0.54, 1.28)**[c]	**<0.001**	**0.71 (0.31, 1.12)**[c]	**0.001**
ICU admission	29 (8.5%)	23 (13.6%)	**1.36 (1.12, 1.67)**[b]	**0.002**	**1.32 (1.07, 1.65)**[b]	**0.01**
TTCS[d], days	4.0 (2.0-7.4)	6.0 (3.0-10.0)	**0.87 (0.80, 0.94)**[a]	**<0.001**	**0.87 (0.80, 0.95)**[a]	**0.002**
CAP complications[e]	73 (30.4%)	53 (44.5%)	**1.23 (1.05, 1.45)**[b]	**0.01**	1.14 (0.96, 1.36)[b]	0.15

Data are median (IQR) or *n* (%) unless otherwise stated and adjusted for age, gender, diabetes mellitus, congestive heart failure and renal insufficiency; FGF21 values were log transformed. [a] Hazard ratio, [b] Odds ratio, [c] Regression coefficient. ICU=intensive care unit.

[d] TTCS Time to clinical stability (n=359; STEP trial) defined as defined as time to clinical stabilization of vital signs at two consecutive measurements ≥ 12 h apart [22].

[e] CAP complications (n=359; STEP trial) defined as recurrence; acute respiratory distress syndrome; empyema; nosocomial infections until day 30; serious adverse events possibly related to CAP; ICU stay; re-admission to hospital.

Table 4 – Prediction of 30-day Mortality: Area under the Receiver Operating Characteristic Curve Plot Analysis

Parameter	AUC	95% CI	*p* Value (vs. FGF21)
Biomarkers			
FGF21	0.73	0.66−0.81	–
C-reactive Protein	0.47	0.37−0.58	**<0.001**
Procalcitonin	0.62	0.53−0.71	**0.03**
White blood cell count	0.31	0.22−0.39	**<0.001**
Established scoring systems			
Pneumonia severity index	0.76	0.66−0.81	0.43
CURB-65	0.60	0.50−0.70	**0.01**

Definition of abbreviations: AUC = area under the curve; CI = confidence interval; FGF21 = Fibroblast growth factor-21.

Table 5 - Association of baseline FGF21 levels with demographic characteristics, comorbidities and clinical variables

	Univariate model		***Multivariate model****	
	Coefficient (95% CI)	***p* value**	**Coefficient (95% CI)**	***p* value**
General characteristics				
Age	8.7 (4.9 12.4)	**<0.001**	-1.5 (-7.1, 4.1)	0.59
Male gender	-171.7 (-310.0, -33.3)	**0.02**	-238.4 (-396.4, -80.3)	**0.003**
BMI	-5.0 (-19.1, 9.0)	0.48	-5.7 (-19.5, 8.1)	0.42
Smoking status	-76.5 (-259.9, 106.9)	0.41	69.8 (-120.9, 260.4)	0.47
Comorbidities				
Diabetes mellitus	167.6 (-4.4, 339.6)	0.06	11.5 (-169.1, 192.1)	0.90
COPD	128.4 (-57.1, 313.8)	0.18	-10.1 (-199.9, 179.8)	0.92
Asthma	-151.6 (-541.0, 237.8)	0.45	3.1 (-374.5, 380.7)	0.99
Heart failure	477.8 (290.4, 665.1)	**<0.001**	226.9 (13.7, 440.1)	**0.04**
Cerebrovascular disease	68.5 (-213.9, 350.9)	0.63	-35.7 (-317.5, 246.1)	0.80
Renal insufficiency	562.7 (416.0, 709.3)	**<0.001**	255.2 (72.0, 438.4)	**0.006**
Neoplastic disease	148.4 (-134.3, 431.1)	0.30	128.6 (-173.9, 431.0)	0.40
Clinical variables				
Body temperature	-24.4 (-106.9, 58.2)	0.56	-4.4 (-82.2, 73.3)	0.91
Systolic blood pressure	-2.5 (-5.8, 0.82)	0.14	-2.3 (-5.8, 1.3)	0.21
Oxygen saturation	-3.0 (-23.2, 17.2)	0.77	-6.7 (-25.2, 11.8)	0.48
Antibiotic pre-treatment	-188.3 (-369.9, -6.8)	**0.04**	-92.5 (-276.2, 91.3)	0.32

Data for univariate and adjusted (multivariate) linear regression analyses are given as regression coefficient (95% CI). BMI, body mass index; CI, confidence interval; COPD, chronic obstructive pulmonary disease; PSI, pneumonia severity index.

*Multivariate model: adjusted for patient age, gender, PSI, antibiotic pre-treatment and comorbidities (diabetes mellitus, congestive heart failure, chronic renal insufficiency).

Figures

Figure legend 1:

Boxplots for levels of FGF21 at baseline stratified by PSI classes (A, C) or by survivors versus non-survivors (B, D) for each trial separately: ProCAP trial left panel (A and B), STEP trial right panel (C and D). Each box signifies the upper and lower quartiles, while the median is represented by a line within the box. Whiskers represent the upper and lower adjacent values, outliers are not depicted. Horizontal dashed red lines with green area represent the 95% confidence interval (CI) of elderly healthy controls (5^{th} percentile 47.5 pg/mL; 95^{th} percentile 343.8 pg/mL). FGF21 discriminates between PSI classes I, II and III versus classes IV and V as the high-risk classes have FGF21 levels clearly above the 95% CI of age-matched controls (A, C). FGF21 levels at baseline potently separated non-survivors from survivors (B, D).

Figure legend 2:

Kaplan-Meier estimators of time-to-clinical stability (A) and length-of-hospital stay (B) for the pooled cohort according to initial FGF21 levels: 3^{rd} tertile (red; >833.3 pg/mL) versus 1^{st} (<258.1 pg/mL) and 2^{nd} (258.2 – 833.33 pg/mL) tertiles (blue). Patients in 3^{rd} tertile of FGF21 levels had significantly prolonged time to clinical stability and length of hospital stay. *p*-values determined by log-rank test.

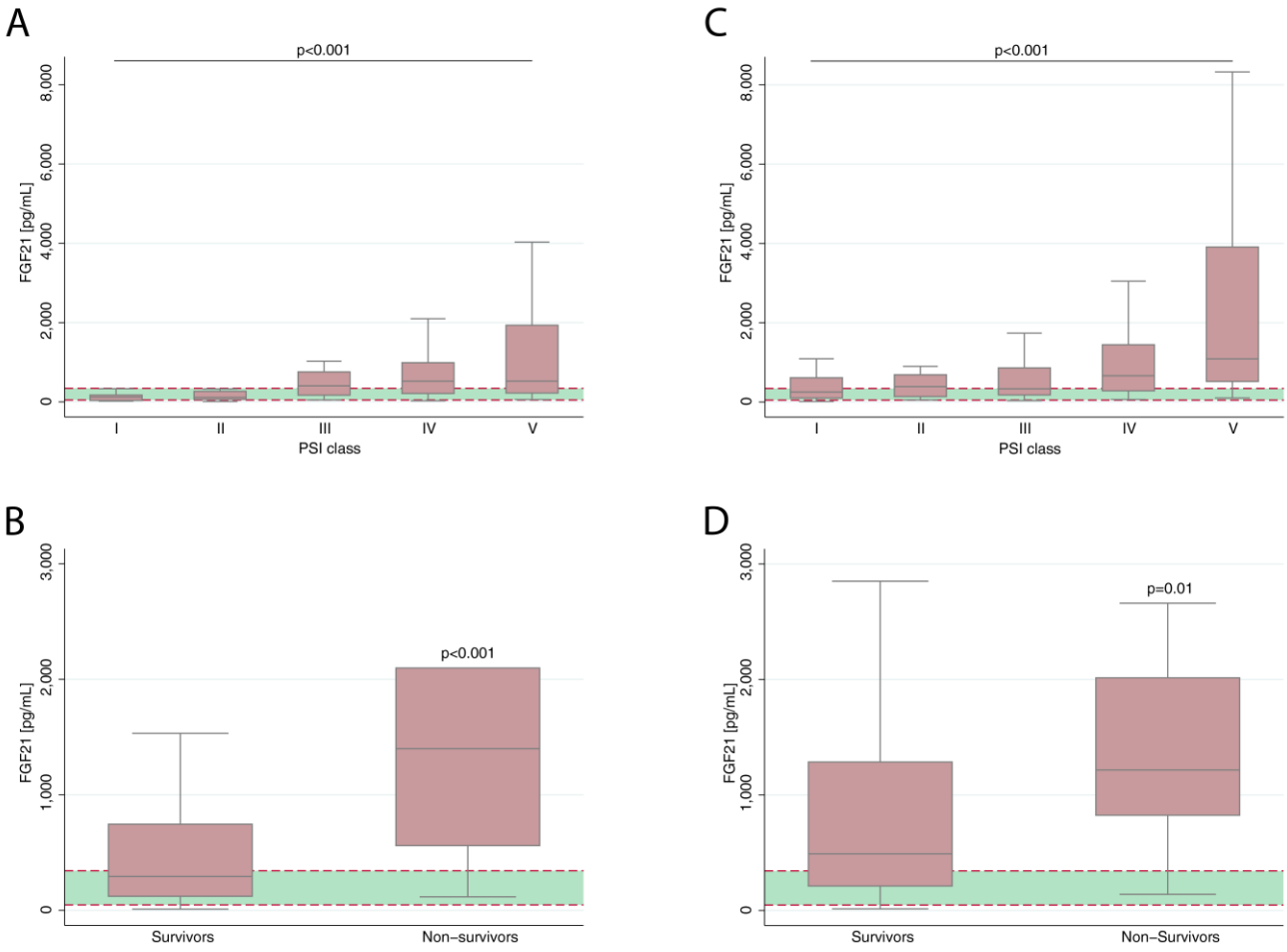
Figure legend 3:

Receiver operating characteristic (ROC) curve analysis of various laboratory parameters versus clinical parameters predicting survival from community-

acquired pneumonia in the pooled cohort. Data on emergency room presentation are shown. Dark-blue line, FGF21 (AUC 0.73); gray line, PSI (AUC 0.76); light-blue line, CURB-65 (AUC 0.60); red line; PCT (AUC 0.62); green line, CRP (AUC 0.47); orange line, WBC (AUC 0.31). AUC = aura under the curve; CRP = C-reactive protein; CURB65 = CURB65 clinical score; FGF21 = Fibroblast Growth Factor-21; PCT = procalcitonin; PSI = pneumonia severity index; WBC = white blood cell count.

Figure legend 4:

Boxplots for change from baseline in FGF21 (deltaFGF21) at day 3 stratified by survivors versus non-survivors for all patients (A), patients randomized to prednisone (B), and patients randomized to placebo (C), respectively. Non-survivors were prone to have an increase in FGF21 levels between day 1 and day 3.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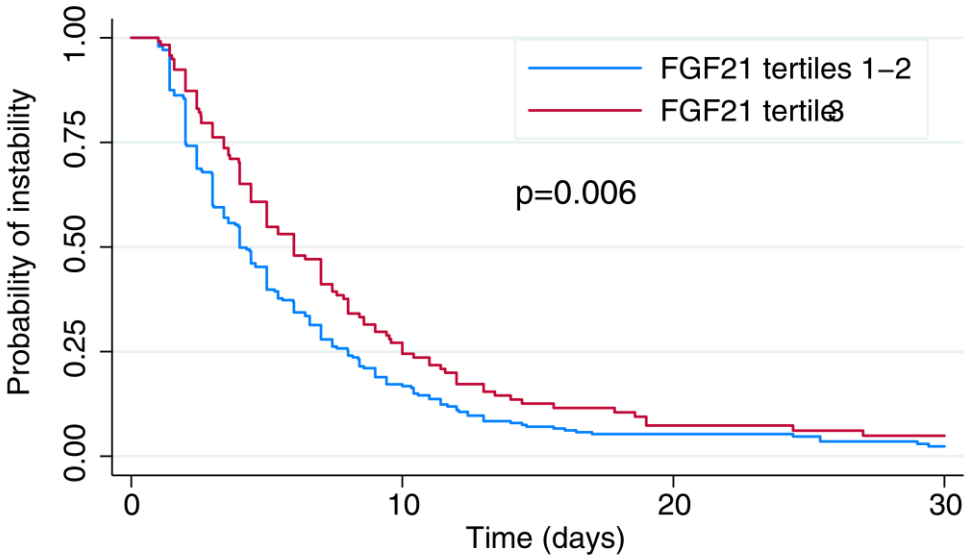
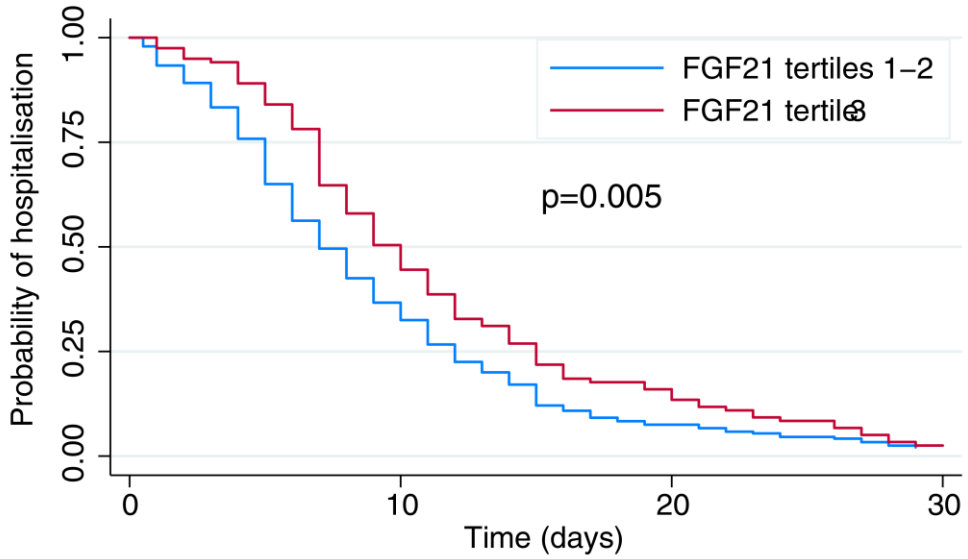
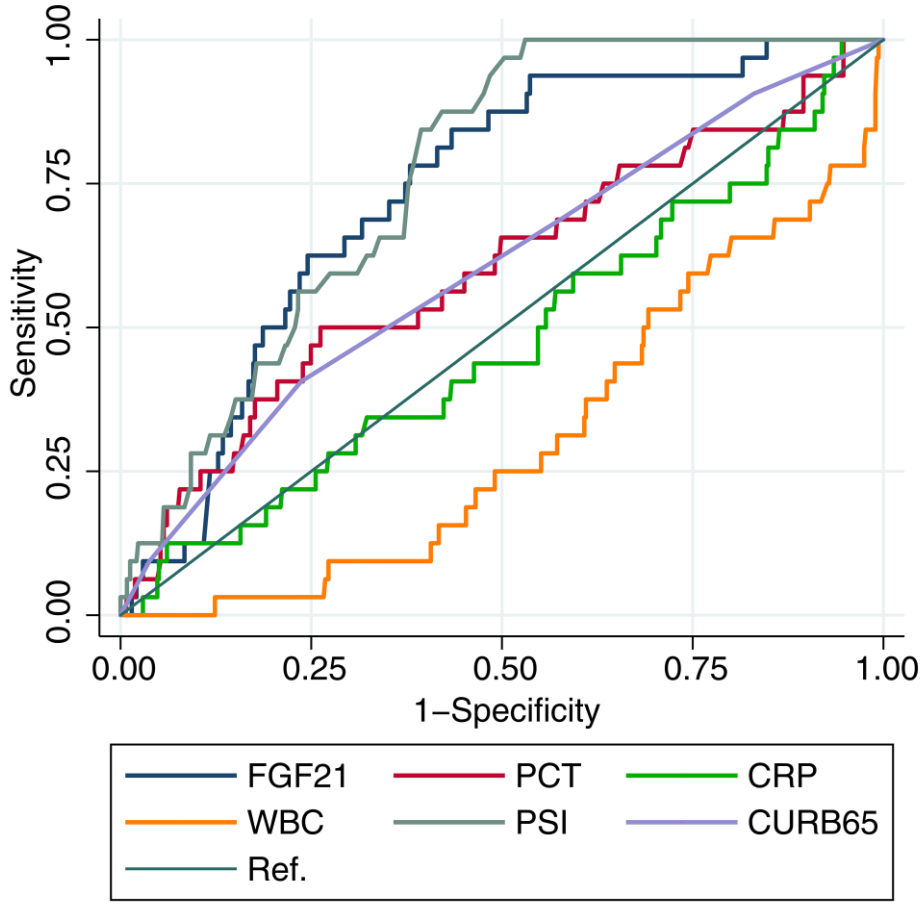


Number at risk				
FGF21 tertiles 1–2	240	40	11	4
FGF21 tertile 3	119	31	7	3

B



Number at risk				
FGF21 tertiles 1–2	240	88	18	5
FGF21 tertile 3	119	60	19	3


Sensitivity
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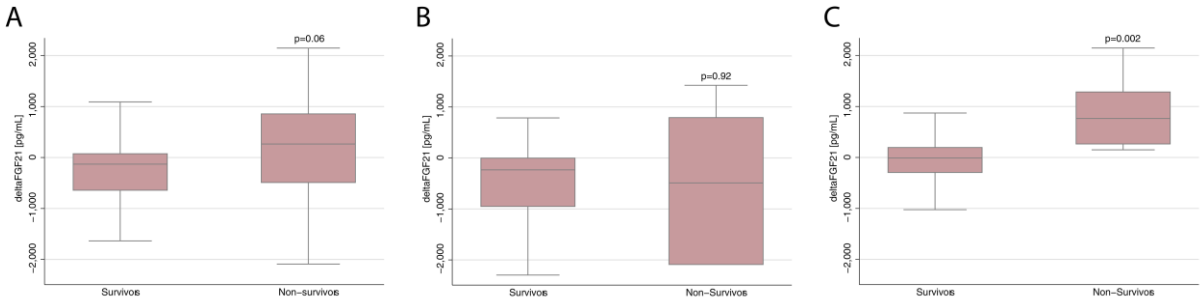
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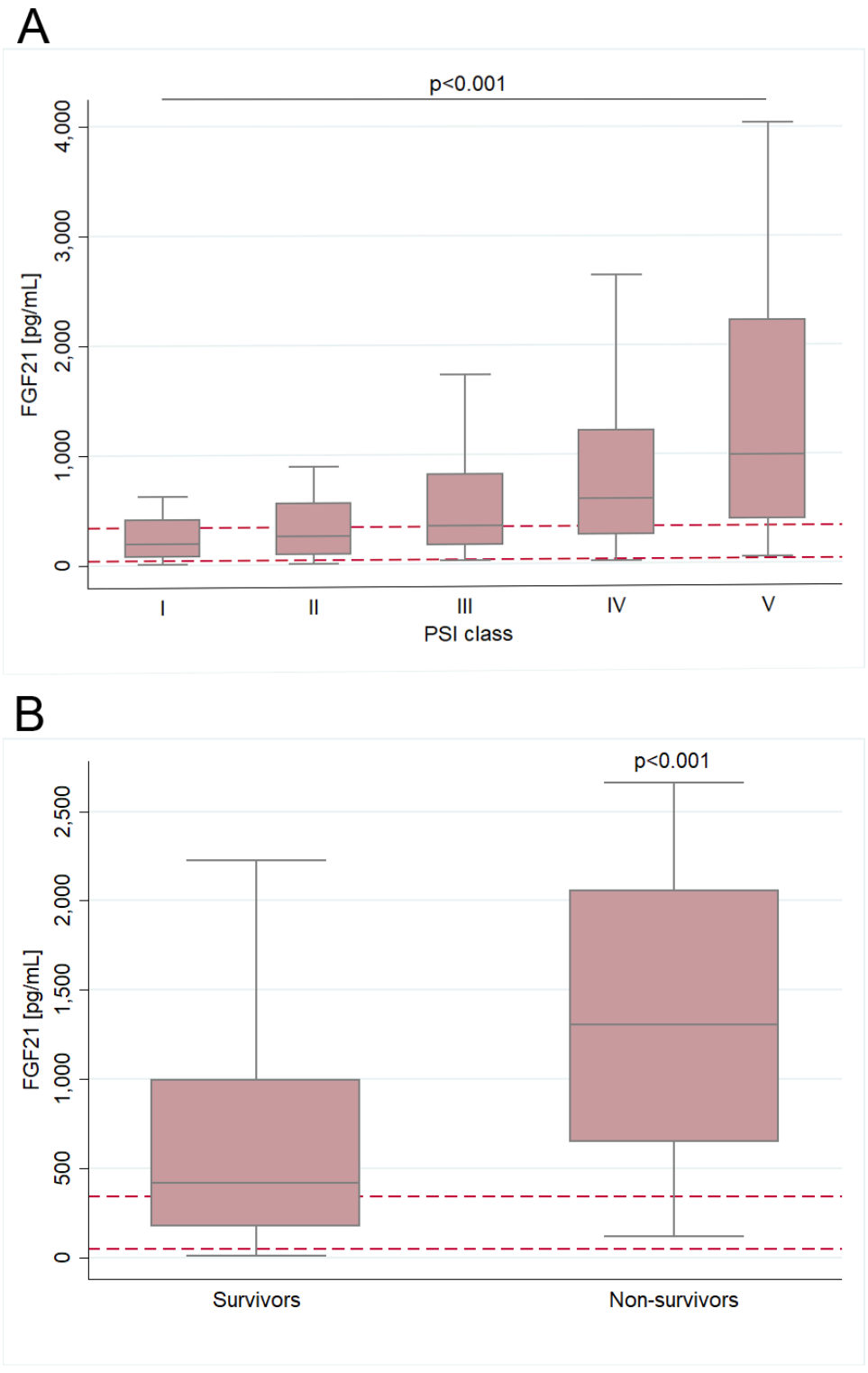
Online Supplemental Material

Fibroblast Growth Factor-21 Predicts Outcome in Community-Acquired Pneumonia

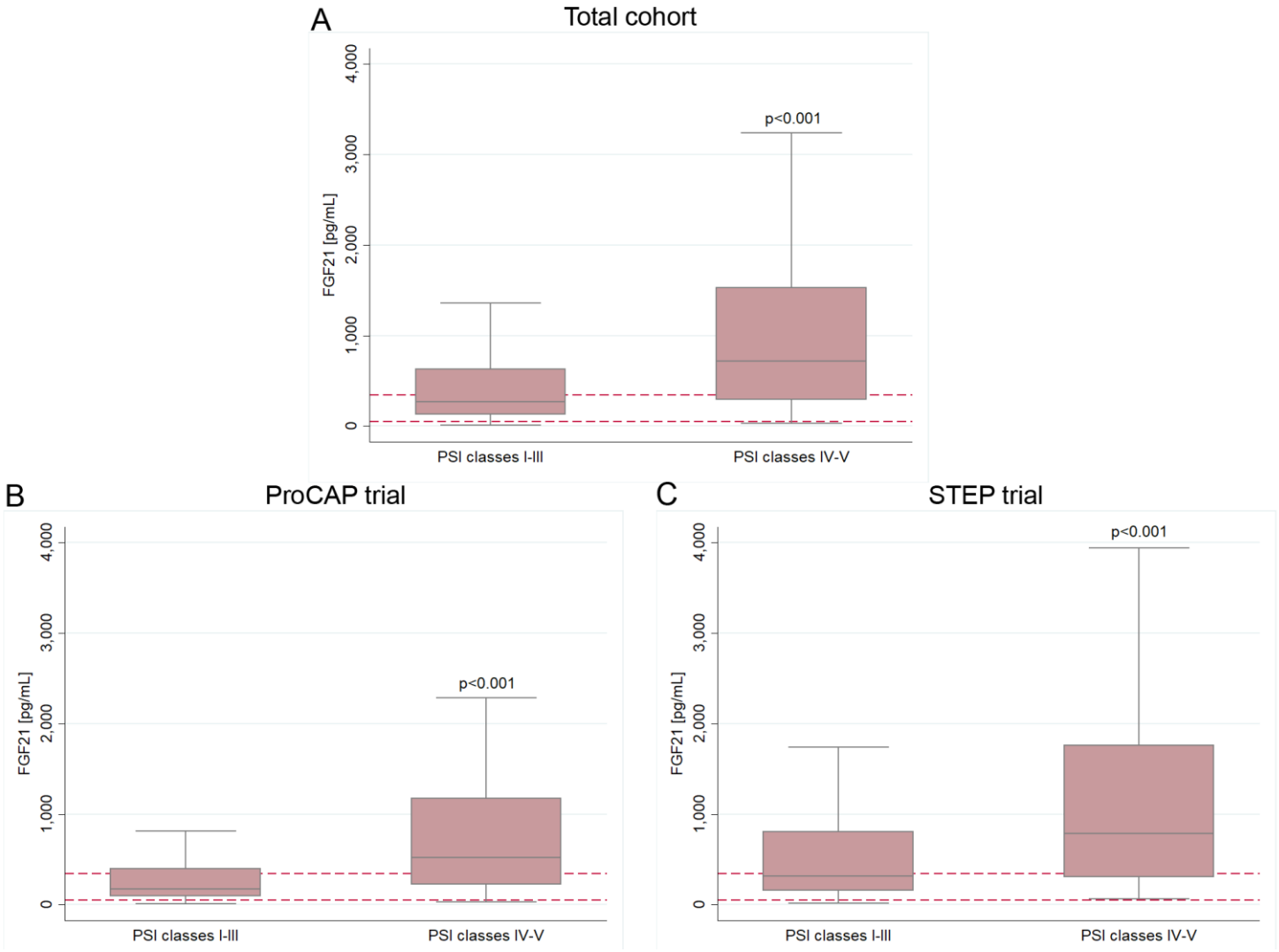
Contents

1. **Figure S1. FGF21 levels and clinical outcomes**



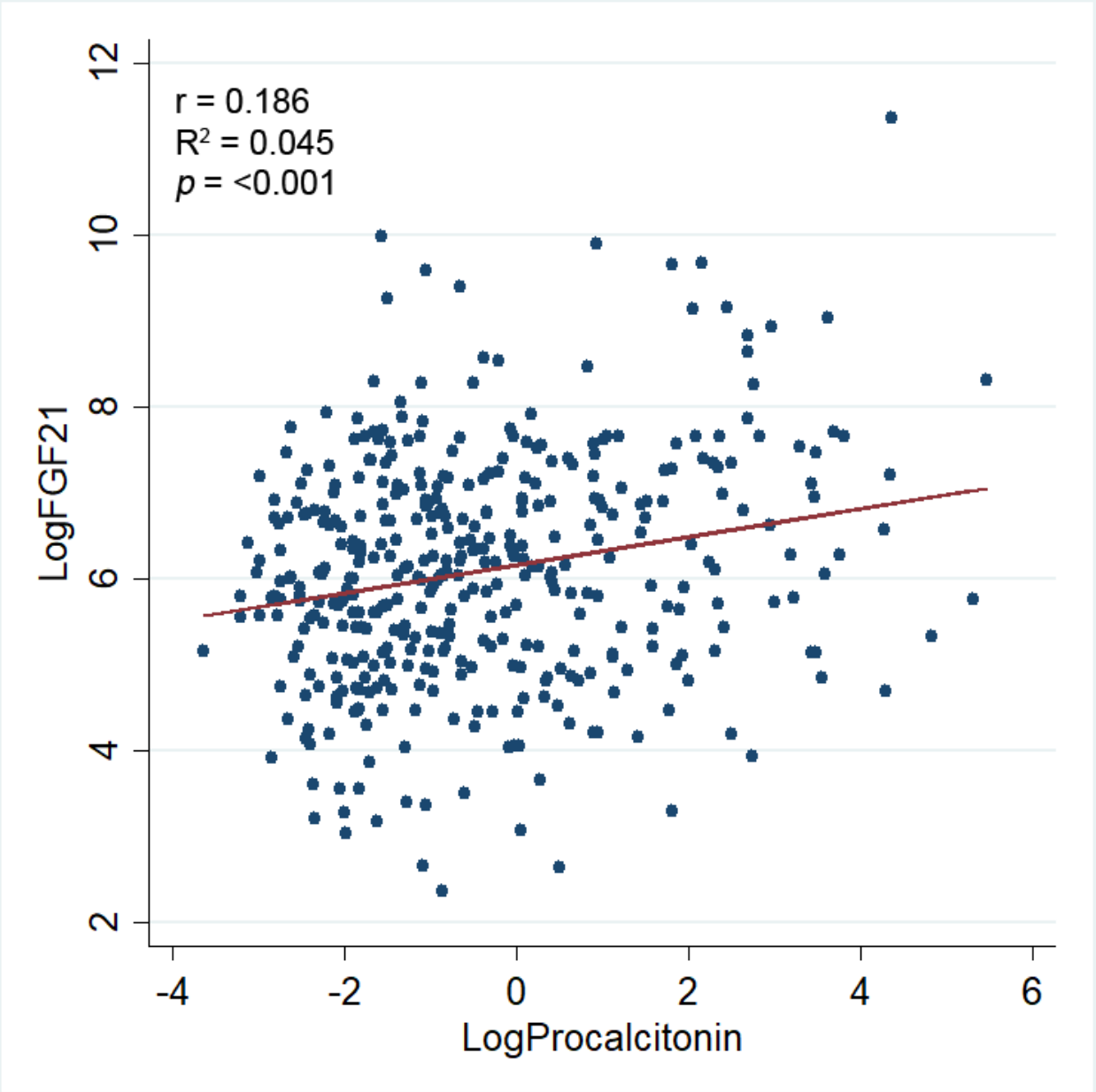
Boxplots for levels of FGF21 at baseline stratified by PSI classes (A) and between Survivors and Non-Survivors (B) for the combined population of ProCAP trial cohort and STEP trial cohort..

2. Figure S2. FGF21 and Pneumonia Severity Index at Emergency Admission



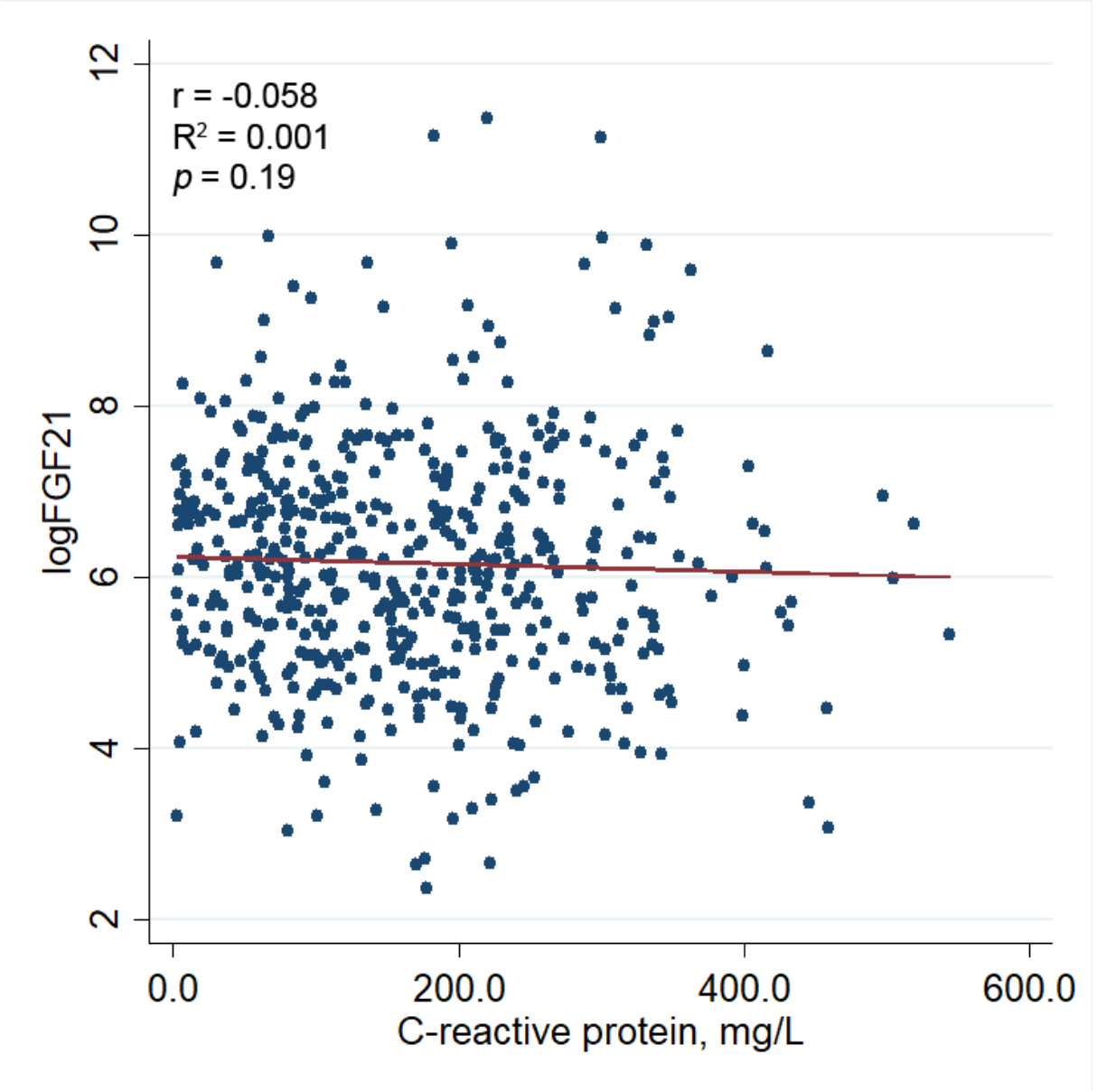
Boxplots for levels of FGF21 at baseline stratified by PSI classes I-III (low-risk) versus PSI classes IV-V (high-risk) for the pooled cohort (A), for the ProCAP trial (B) and for the STEP trial cohort (C).

3. **Figure S3. FGF21 and Procalcitonin at Emergency Admission**



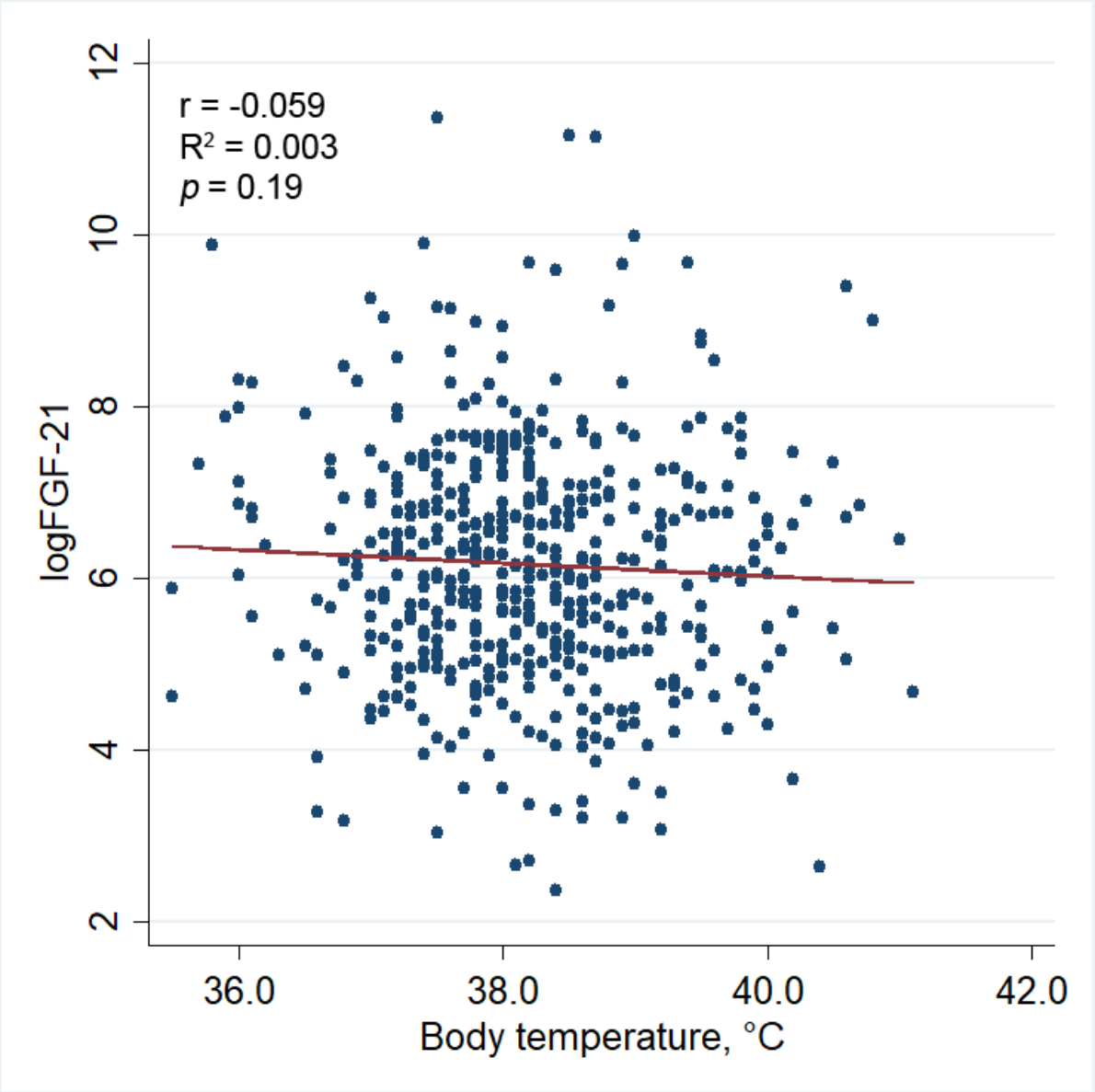
Scatter plot with fitted linear regression line for baseline levels of log-transformed Fibroblast Growth Factor-21 (FGF21) and log-transformed procalcitonin levels at emergency admission. There is a significant correlation between these two inflammatory parameters (r=0.186, R^2 0.045; p<0.001).

4. **Figure S4. FGF21 and C-reactive protein at Emergency Admission**



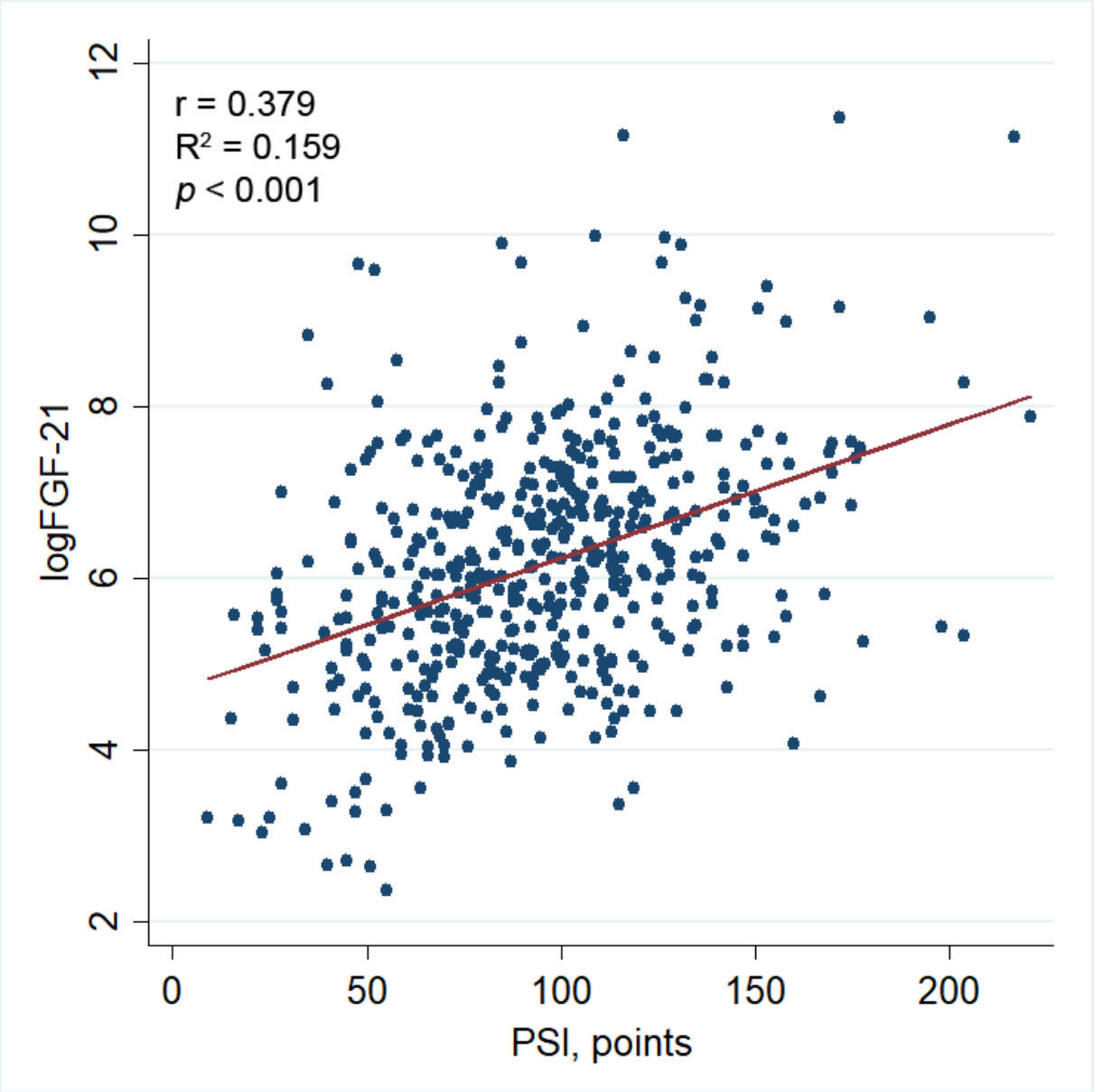
Scatter plot with fitted linear regression line for baseline levels of log-transformed Fibroblast Growth Factor-21 (FGF21) and c-reactive protein levels at emergency admission. There is no significant correlation between these parameters.

5. Figure S5. FGF21 and Body Temperature at Emergency Admission



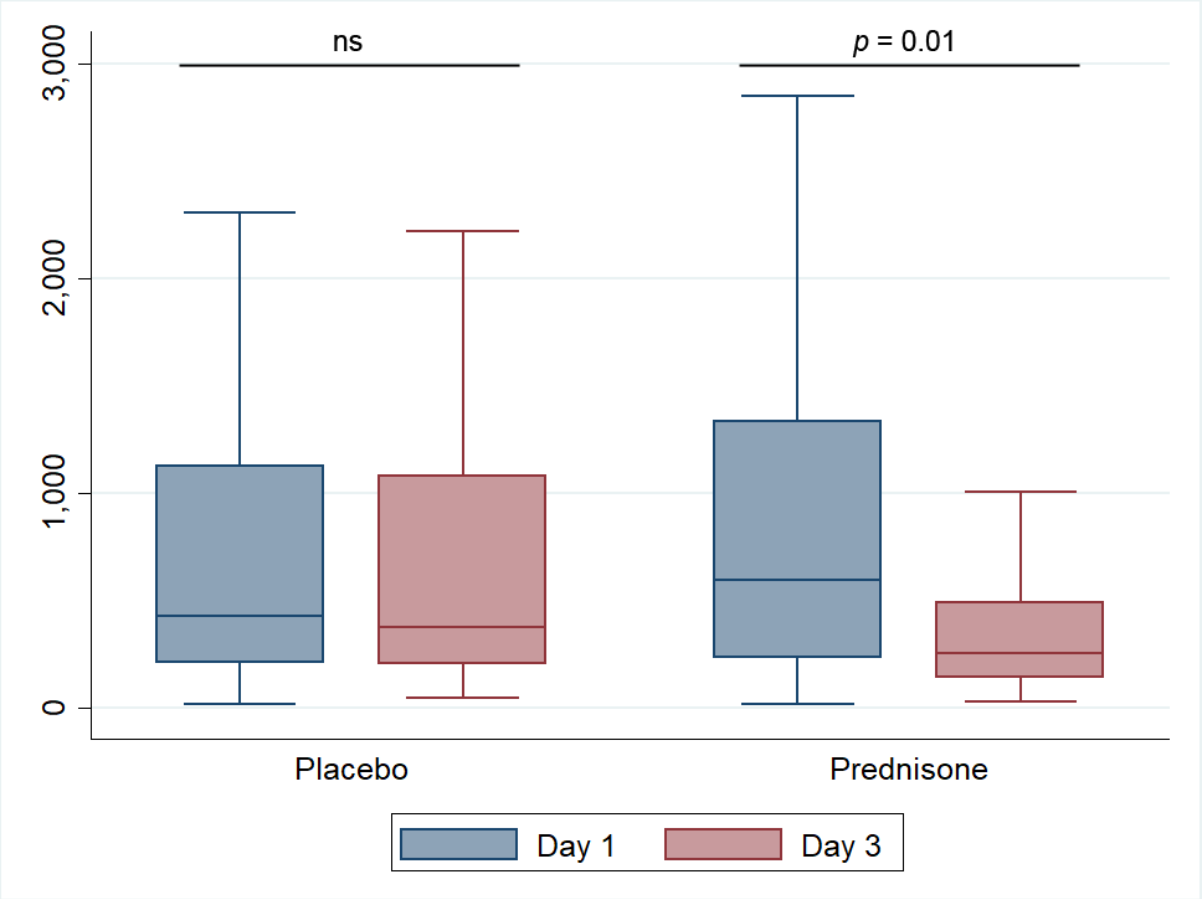
Scatter plot with fitted linear regression line for baseline levels of log-transformed Fibroblast Growth Factor-21 (FGF21) and body temperature at emergency admission. There is no significant correlation between these parameters.

6. Figure S6. FGF21 and Pneumonia Severity Index (PSI)



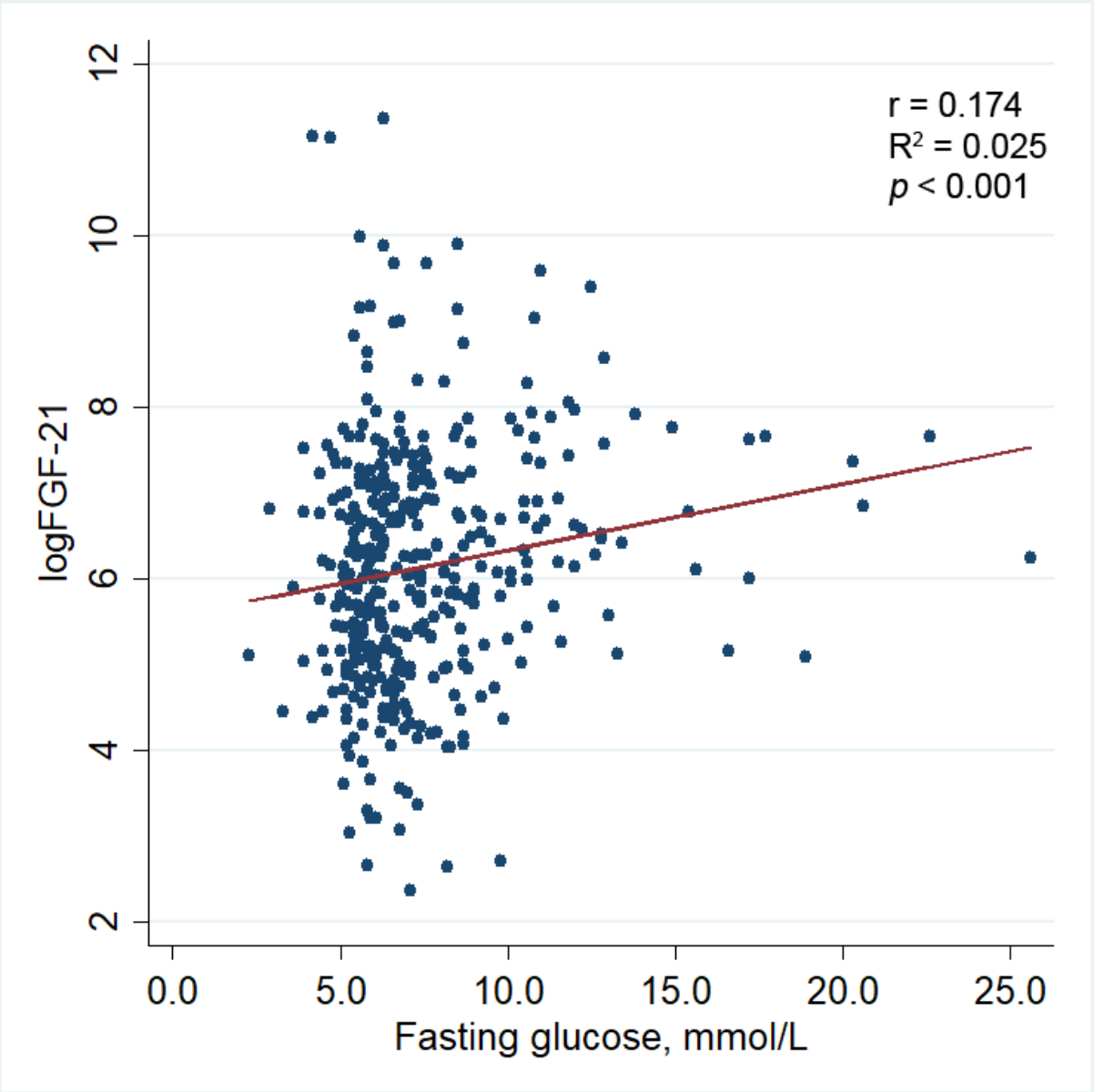
Scatter plot with fitted linear regression line for baseline levels of log-transformed Fibroblast Growth Factor-21 (FGF21) and Pneumonia Severity Index (PSI) of the pooled total cohort (ProCAP and STEP trials), revealing a significant positive correlation of FGF21 with disease severity.

7. Figure S7. Effects of Corticosteroids on FGF21 levels



Box plots for levels of Fibroblast Growth Factor-21 (FGF21 in pg/mL) at emergency admission (blue) and day 3 (red) stratified by randomized treatment (Placebo vs. Prednisone). There was a significant decrease in FGF21 levels at day 3 upon treatment with prednisone 50mg per day.

8. **Figure S8. FGF21 and Glucose levels at Emergency Admission**



Scatter plot with fitted linear regression line for baseline levels of log-transformed Fibroblast Growth Factor-21 (FGF21) and glucose levels at emergency admission of the pooled total cohort (ProCAP and STEP trials), revealing a significant positive correlation of FGF21 with plasma glucose.

9. Table S1. Baseline characteristics of the ProCAP and STEP cohorts

Characteristic/variable	Total cohort (n=509)	ProCAP trial (n=150)	STEP trial (n=359)
General characteristics			
Age, years	75 (61, 83)	73.0 (56.0, 82.0)	75 (62, 83)
Male sex	320 (62.9%)	93 (62.0%)	227 (63.2%)
BMI, kg/m^2	26.0 (23.0, 29.3)	24.8 (22.2, 28.7)	26.1 (23.0, 29.4)
Smoking status	118 (23.2%)	32 (21.3%)	86 (24.0%)
Packyears, years	20.0 (0.0, 40.0)	30.0 (20.0, 50.0)	10.0 (0.0, 40.0)
Comorbidities			
Diabetes mellitus	117 (23.0%)	35 (23.3%)	82 (22.8%)
COPD	100 (19.6%)	32 (21.3%)	68 (18.9%)
Asthma	21 (4.1%)	3 (2.0%)	18 (5.0%)
Heart failure	83 (16.3%)	9 (6.0%)	74 (20.6%)
Cerebrovascular disease	44 (8.6%)	9 (6.0%)	35 (9.7%)
Renal insufficiency	165 (32.4%)	34 (22.7%)	131 (36.5%)
Neoplastic disease	41 (8.1%)	23 (15.3%)	18 (5.0%)
Antibiotic pre-treatment	109 (21.4%)	31 (20.7%)	78 (21.7%)
Clinical variables			
Systolic blood pressure, mmHg	125.0 (110.0, 140.0)	130.0 (113.0, 142.0)	124.0 (110.0, 140.0)
Heart rate, bpm	88.0 (76.0, 101.0)	96.0 (84.0, 108.0)	85.0 (74.0, 98.0)
Respiratory rate, breaths/min	21.0 (18.0, 25.0)	22.0 (18.0, 27.0)	20.0 (18.0, 24.0)
Body temperature, °C [in-ear]	38.1 (37.5, 38.7)	38.5 (37.7, 39.3)	38.0 (37.4, 38.6)
SIRS, points.	2 (2, 3)	3 (2, 4)	2 (2, 3)
PSI class†			
I, II and III	227 (44.6%)	64 (42.7%)	163 (45.4%)
IV and V	282 (55.4%)	86 (57.3%)	196 (54.6%)
PSI, points†	95 (71, 118)	95 (71, 115)	95 (71, 118)
Laboratory values			
C-reactive protein, mg/L	153.0 (80.0, 233.0)	135.5 (74.0, 216.4)	153.6 (80.1, 240.0)
Procalcitonin, ng/dL	0.4 (0.2, 1.9)	0.4 (0.2, 1.4)	0.4 (0.2, 2.5)
White blood cell count, G/L	12.2 (8.8, 15.4)	12.8 (9.0, 15.4)	12.0 (8.7, 15.4)
Fasting glucose, mmol/L	6.6 (5.7, 8.4)	6.8 (5.8, 8.7)	6.6 (5.7, 7.8)

Data are presented as median (interquartile range) or n (%), unless otherwise stated. BMI: body mass index; COPD: chronic obstructive pulmonary disease; SIRS: systemic inflammatory response syndrome; PSI: Pneumonia Severity Index. † The PSI is a clinical prediction rule to calculate the probability of morbidity and mortality in patients with community-acquired pneumonia [1]; PSI risk class I corresponds to age ⩽50 years and no risk factors (⩽50 points), risk class II to 51–70 points, risk class III to 71–90 points, risk class IV to 91–130 points and risk class V to >130 points.

10. Table S2a. Baseline characteristics of young healthy volunteers

Characteristic/variable		**Total cohort (n=56)**
Sex	Female	11 (20%)
	Male	45 (80%)
Age, years		24 (22, 29.5)
Weight, kg		74.5 (68.5, 79.1)
Height, cm		179.5 (173, 183)
BMI, kg/m^2		23.1 (21.5, 24.7)
FGF21, pg/mL		50.4 (13.7, 113.3)

Data are presented as median (IQR) for continuous or n (%) for categorical variables.
BMI, body mass index.

11. Table S2b. Baseline characteristics of older healthy volunteers

Characteristic/variable		**Total cohort (n=56)**
Sex	Female	28 (70%)
	Male	12 (30%)
Age, years		64 (59.5, 71.5)
Weight, kg		64.5 (57.5, 78.1)
Height, cm		166 (160, 169)
BMI, kg/m^2		23.4 (21.3, 26.2)
C-reactive protein, mg/L		1.0 (0.4, 1.6)
FGF21, pg/mL		140.2 (81.1, 161.8)

Data are presented as median (IQR) for continuous or n (%) for categorical variables.
BMI, body mass index.

12. Table S3. FGF21 and Pneumonia severity index to predict 30-day mortality: Sensitivity, Specificity, Positive likelihood ratio, and negative likelihood ratio at various cutoff values

Cutoff	Sensitivity	Specificity	LR+	LR-
FGF21				
386	90.6	46.8	1.7	0.2
669	75.0	62.7	2.0	0.4
1933	31.3	86.6	2.3	0.8
PSI				
95	90.6	52.4	1.9	0.2
102	84.4	60.6	2.1	0.3
134	31.3	87.6	2.5	0.8

Definition of abbreviations: FGF21 = Fibroblast growth factor-21; LR+ = positive likelihood ratio; LR– = negative likelihood ratio; PSI = pneumonia severity index.

13. Table S4. Diagnostic accuracy to discriminate among low-risk (PSI I-III) versus high-risk (PSI IV-V) in community-acquired pneumonia

Parameter	AUC	95% CI	*p* Value (vs. FGF21)
FGF21	0.68	0.64−0.73	–
C-reactive Protein	0.48	0.43−0.53	<0.001
Procalcitonin	0.56	0.51−0.62	0.02
White blood cell count	0.46	0.41−0.51	<0.001

Definition of abbreviations: AUC = area under the curve; CI = confidence interval; FGF21 = Fibroblast growth factor-21; PSI = pneumonia severity index.

14. Table S5. FGF21 and clinical outcomes in STEP trial

			Regression Analysis of Baseline FGF21			
	Tertiles 1-2 n=240	Tertile 3 n=119	Univariate regression analysis, OR, HR, coefficient (95% CI)	*p* value	Multivariable adjusted regression analysis, OR, HR, coefficient (95% CI)	*p* value
Death (30-days)	5 (2.1%)	10 (8.4%)	**1.53 (1.09, 2.14)**	**0.01**	1.53 (0.99, 2.36)	0.05
Time to effective hospital discharge, days	7.0 (6.0-8.0)	10.0 (8.0-11.0)	**0.85 (0.78, 0.92)**	**<0.001**	**0.87 (0.80, 0.95)**	**0.001**
Total duration of antibiotic treatment, days	10.0 (8.0-13.0)	11.0 (8.0-14.0)	**0.54 (0.11, 0.98)**	**0.01**	0.35 (-0.12, 0.82)	0.15
Intravenous antibiotic treatment, days	5.0 (3.0-7.0)	6.0 (5.0-9.0)	**0.97 (0.55, 1.40)**	**<0.001**	**0.78 (0.31, 1.24)**	**0.001**
ICU admission	17 (7.1%)	17 (14.3%)	**1.45 (1.14, 1.85)**	**0.003**	**1.39 (1.06, 1.81)**	**0.02**
TTCS[d], days	4.0 (2.0-7.4)	6.0 (3.0-10.0)	**0.87 (0.80, 0.94)**[a]	**<0.001**	**0.87 (0.80, 0.95)**[a]	**0.002**
CAP complications[e]	73 (30.4%)	53 (44.5%)	**1.23 (1.05, 1.45)**[b]	**0.01**	1.14 (0.96, 1.36)[b]	0.15

Data are median (IQR) or *n* (%) unless otherwise stated and adjusted for age, gender, diabetes mellitus, congestive heart failure and renal insufficiency; FGF21 values were log transformed. [a] Hazard ratio, [b] Odds ratio, [c] Regression coefficient. ICU=intensive care unit.

[d] TTCS Time to clinical stability defined as defined as time to clinical stabilization of vital signs at two consecutive measurements ≥ 12 h apart.

[e] CAP complications defined as recurrence; acute respiratory distress syndrome; empyema; nosocomial infections until day 30; serious adverse events possibly related to CAP; ICU stay; re-admission to hospital.

15. Table S6. FGF21 clinical outcomes in ProCAP trial

			Regression Analysis of Baseline FGF21			
	Tertiles 1-2 n=100	Tertile 3 n=50	Univariate regression analysis, OR, HR, coefficient (95% CI)	*p* value	Multivariable adjusted regression analysis, OR, HR, coefficient (95% CI)	*p* value
Death (30-days)	5 (5.0%)	12 (24.0%)	**2.31 (1.45, 3.69)**	**<0.001**	**2.13 (1.30, 3.48)**	**0.002**

Data are median (IQR) or *n* (%) unless otherwise stated and adjusted for age, gender, diabetes mellitus, congestive heart failure and renal insufficiency; FGF21 values were log transformed. [a] Hazard ratio, [b] Odds ratio, [c] Regression coefficient. ICU=intensive care unit.

Time to effective hospital discharge, days	11.0 (9.0-15.0)	13.0 (10.0-14.0)	0.91 (0.79, 1.03)	0.13	0.90 (0.79, 1.03)	0.13
Total duration of antibiotic treatment, days	9.0 (5.0-13.0)	10.0 (4.0-14.0)	0.84 (-0.39, 1.72)	0.06	1.00 (0.01, 1.99)	0.05
Intravenous antibiotic treatment, days	4.5 (3.0-6.0)	4.5 (3.0-6.5)	0.12 (-0.64, 0.60)	0.96	0.18 (-0.61, 0.98)	0.64
ICU admission	13 (13.0%)	7 (14.0%)	1.30 (0.89, 1.88)	0.18	1.49 (0.97, 2.28)	0.07

16. References

1 Fine MJ, Auble TE, Yealy DM, Hanusa BH, Weissfeld LA, Singer DE, Coley CM, Marrie TJ, Kapoor WN. A prediction rule to identify low-risk patients with community-acquired pneumonia. *The New England journal of medicine* 1997; 336: 243–250.