Sex-specific Effect and Disconnect Between Plasma Levels of FXII and FXIIa-C1-esterase Inhibitor Complexes in Pneumonia

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Abstract

Background: Sex-dependent differences in immunity and coagulation play an active role in the outcome of community-acquired pneumonia (CAP). Factor XII acts at the crossroads between inflammation and coagulation thus representing a point of convergence in host defense against infection. Here, we evaluated FXII/FXIIa levels in plasma of CAP patients and correlated them to clinical disease severity.

Methods and Results: FXII was activated in CAP plasma as evident by the presence of FXIIa-C1-esterase inhibitor (C1INH) complexes. The levels of FXIIa-C1INH complexes were elevated in plasma of CAP patients (n=140) as compared to age- and sex-matched healthy controls (n=58; $p<0.001$). No simultaneous decrease in FXII levels, indicating its consumption, was observed. Stratification by sex revealed augmented levels of FXII in plasma of CAP women as compared to healthy females ($p=0.008$) yet no apparent differences in men ($p=0.619$). This sex-specific difference was, however, attributable to the lower levels of FXII in healthy females relative to healthy men ($p=0.011$). Upon contact with CAP plasma, isolated blood neutrophils released FXII and female blood neutrophils were able to re-induce FXII mRNA synthesis ($p=0.031$). Despite this sex-specific effect, exposure of female blood neutrophils to estradiol did not induce FXII mRNA expression. Finally, although we observed accumulation of FXIIa-C1INH complexes in plasma of severe CAP patients, the relationship between the levels of FXIIa-C1INH complexes and CAP severity, as assessed by CRB65 score, did not reach statistical significance ($p=0.057$).

Conclusions: Our study identifies age- and sex-dependent differences in FXII expression that may contribute to specific clinical outcomes in CAP in different patient subgroups.

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Figures

Figure 1

The levels of FXII in plasma of women and men suffering from CAP. A) Western blot analysis of FXII and FXIIa in plasma from CAP patients and donors (healthy subjects). Six patients with CAP and 6 donors were analyzed. Purified FXII and FXIIa were used as positive controls. Coomassie Brilliant Blue (CBB) stained membrane is shown to demonstrate equal loading. B, C) Levels of FXII in plasma of donors and CAP patients as assessed by ELISA ((B) by group; (C) by group and sex). D) Correlation between the levels of FXII in plasma of donor and CAP women and age. For all: red represents women and black men.

CAP, community acquired pneumonia; FXII, factor XII; FXIIa, activated FXII; pFXII(a), purified FXII(a); Hch, heavy chain; Lch, light chain.

Figure 2

FXII gender-specific effects in CAP. A) Correlation between the levels of FXII and the concentration of CRP on admission in plasma of CAP patients . B) FXII levels and CRB65 score values on admission in patients suffering from CAP. C) 28- day survival probability in dependency of FXII levels in plasma of CAP patients. For all: red represents women and black men. CAP, community acquired pneumonia; FXII, factor XII; CRP, C-reactive protein.

Figure 3

Activation of FXII in CAP plasma. A) Western blot analysis of FXIIa-C1INH complexes in plasma from CAP patients and donors (healthy subjects). FXIIa-C1INH complexes were detected using the RII antibody recognizing free C1INH and C1INH in complex with FXIIa. Lane labeled "pFXIIa" is loaded with purified FXIIa, lane labelled "pC1INH" with purified C1INH, and lane labelled with pFXIIa+pC1INH with a mixture of both. One hundred forty patients with CAP and 57 donors were analyzed. Five CAP patients and 5 donors are demonstrated. B) Western blot analysis of PKa in donor and CAP plasma samples. Six CAP patients and 6 donors were analyzed. C) Western blot analysis of HK in donors and CAP plasma samples. Intact HK and cleaved HK (light chain) are shown. Six CAP patients and 6 donors were analyzed. Coomassie Brilliant Blue (CBB) stained membranes are shown to demonstrate equal loading. * indicates unspecific bands. CAP, community acquired pneumonia; FXII, factor XII; FXIIa, activated FXII; pFXIIa, purified; C1 esterase inhibitor (C1INH); HK, high molecular weight kininogen; cHK, cleaved HK; PKa, plasma kallikrein; Hch, heavy chain; Lch, light chain.

Figure 4

The plasma levels of FXIIa-C1INH complexes and C1INH alone in CAP women and men. A) Western blot analysis of FXIIa-C1INH complexes in plasma from CAP patients and donors. FXIIa-C1INH complexes were detected using the RII antibody recognizing free C1INH and C1INH in complex with FXIIa. Albumin was used as a loading control. Ten out of 140 CAP patients and 4 out of 57 donors are demonstrated. B-D) Data obtained from densitometric analysis of the FXIIa-C1INH complex by group (B) and by group and sex (C) and of C1INH by group and sex (D). For all: red represents women and black men. CAP, community acquired pneumonia; FXIIa, activated factor XII; C1INH, C1 esterase inhibitor.

Figure 5

FXIIa-C1INH complexes accumulate in severe CAP. A) Correlation between the levels of FXIIa-C1INH complexes and the concentration of CRP in plasma on admission in CAP patients (n=140). B) Western blot analysis of FXIIa-C1INH complexes in plasma from CAP patients with different CRB65 scores. FXIIa-C1INH complexes were detected using the RII antibody recognizing free C1INH and C1INH in complex with FXIIa. Albumin was used as a loading control. Fourteen CAP patients are shown. C) The levels of FXIIa-C1INH complexes and CRB65 score values on admission in CAP patients (CRB65 score 0, n=27; CRB65 score 1, n=61; CRB65 score 2, n=29; CRB65 score 3, n=20). D) 28-day survival probability in dependency of the levels of FXII-C1INH complexes in plasma of CAP patients. For all: red represents

women and black men. CAP, community acquired pneumonia; FXIIa, activated factor XII; C1INH, C1 esterase inhibitor; CRP, C-reactive protein.

Figure 6

Changes in FXII/FXIIa levels in CAP lungs. A) Western blot analysis of FXII (upper panel) and FXIIa-C1INH complexes (middle panel) in BAL from CAP patients and donors. Ten patients with CAP and 20 donors were analyzed. Purified FXII was used as positive controls. Five pneumonia patients and 5 donors are

demonstrated. Albumin served as a loading control (lower panel). B, C) Levels of FXII B) and FXIIa C) in BAL of donors and CAP patients as assessed by ELISA. D) Levels of FXIIa-C1INH complexes as assessed by densitometry analysis of the middle panel presented in A). E) mRNA levels of FXII in CAP and donor lung tissue. The qPCR data are presented as ΔCT using PBGD as a reference gene. F) Immunolocalisation of FXII in CAP lungs. To identify FXII positive cells the serial section were stained for proSP-C (marker of ATII cells, shown with an open head arrow), CD68 (marker for alveolar macrophages, shown with a star), and vWF (marker for endothelial cells, shown with a closed head arrow). CAP, community acquired pneumonia; FXII, factor XII; FXIIa, activated FXII; pFXII, purified FXII; C1INH, C1 esterase inhibitor; proSP-C, prosurfactant protein-C; vWF, von Willebrand factor; Hch, heavy chain.

Figure 7

Women neutrophils re-express FXII under CAP condition. A) FXII mRNA levels in blood neutrophils exposed to CAP or donor (healthy subject) plasma. n=10. B) Gender-specific expression of FXII mRNA in blood neutrophils upon exposure to CAP or donor plasma. n=6/5 (female/male). C) FXII mRNA levels in neutrophils either untreated or treated for 4h with 1 nM 17 β -estradiol n=3/3 (female/male). All qPCR data are presented as ΔCT using PBGD as a reference gene. Biological replicates are demonstrated. D) Numbers of neutrophils on admission in CAP men and women. Clinical data was not available for all CAP patients. For all: red represents women and black men. E) Immunolocalisation of FXII in neutrophils exposed to CAP or donor plasma. Blood neutrophils isolated from a FXII deficient subject served as a staining control. Bar size 10 μm . n=10 biological replicates.. CAP, community acquired pneumonia; FXII, factor XII.

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