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New antibodies for dPAPP-A immunoassays

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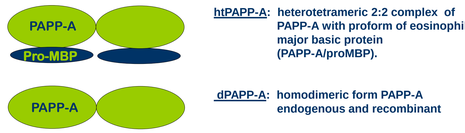
Introduction

Pregnancy associated plasma protein (PAPP-A) is a high-molecular weight glycoprotein initially identified in blood of pregnant women as heterotetrameric htPAPP-A complex with proform of eosinophil major basic protein (PAPP-A/proMBP). Recently it was revealed that PAPP-A is also expressed in unstable atherosclerotic plaques and can be used as a blood **marker** of adverse outcome in acute coronary syndrome (ACS) patients. Plaque form of PAPP-A was shown to be homodimeric **dPAPP-A** (not complexed with the proMBP).

So far, immunoassays that do not discriminate htPAPP-A and dPAPP-A have been used for dPAPP-A measurements in blood of ACS patients. However, low but appreciable levels of htPAPP-A in normal and ACS patients' blood could significantly influence on the results of dPAPP-A measurements by these non-specific assays.

Thus, **dPAPP-A-specific** immunoassay w/o crossreaction with htPAPP-A seems to be very promising tool for accurate marker quantification.



htPAPP-A: heterotetrameric 2:2 complex of PAPP-A with proform of eosinophil major basic protein (PAPP-A/proMBP).

dPAPP-A: homodimeric form PAPP-A endogenous and recombinant

The aim of our study was to generate monoclonal antibodies (MAb) specific to dPAPP-A and to design the prototype immunoassays suitable for dPAPP-A measurements in ACS patients' blood.

Materials and Methods

Monoclonal antibodies:

After BALB/c mice immunization with dPAPP-A purified from human atherosclerotic coronary vessels we have obtained several hybridoma cell lines producing monoclonal antibodies, specific to endogenous dPAPP-A (or recombinant dPAPP-A, produced in mammalian cell line) and having no cross-reaction with htPAPP-A.

All new MAbs were tested in pairs as capture and detection antibodies with MAbs able to recognize all three forms of PAPP-A: htPAPP-A, endogenous dPAPP-A and recombinant dPAPP-A (HyTest, Finland). The best out of 300 antibody combinations were selected for further analysis.

Sandwich immunofluorescent assay (IFA):

In developed sandwich IFAs:

- one antibody was specific to dPAPP-A (without cross-reaction with htPAPP-A),
- whereas another one was able to recognize all three forms of PAPP-A.

Detection antibodies were labeled with Eu(3+)-chelate. The fluorescence was measured by Victor 1420 multilabel counter (Wallac-Percin Elmer, Finland).

Results and Discussion

Out of 300 tested two-site combinations two pairs **PAPP8-PAPP24** and **PAPP52-PAPP30** (capture-detection) were selected for the development of dPAPP-A specific sandwich immunoassays. In contrast to previous generations of PAPP-A immunoassays that recognized both dPAPP-A and htPAPP-A (Fig.1, B), our new immunoassays were able to recognize dPAPP-A only (Fig.1, A).

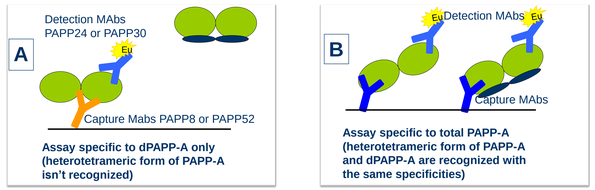


Figure 1. Schematic representation of sandwich immunofluorescent assays used in the study for measurements:
- dPAPP-A only **(A)**
- total PAPP-A **(B)**

In new assays one antibody (MAb PAPP8 or MAb PAPP30) was specific to dPAPP-A (without cross-reaction with htPAPP-A), whereas another one (MAb PAPP24 or MAb PAPP52) was able to recognize all three forms of PAPP-A. Detection MAbs PAPP24 and PAPP30 were labeled with stable Eu(3+) chelate.

Both assays recognized endogenous and recombinant dPAPP-A and showed very low cross-reactivity (< 1%) with htPAPP-A.

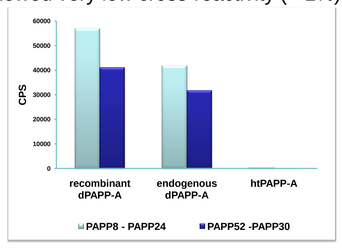


Figure 2. Immunoreactivity studies of three forms of PAPP-A in new immunoassays.

The analytical detection limit of assays was less than 1 ng/mL, when purified recombinant dPAPP-A (HyTest, Finland) was used as a calibrator.

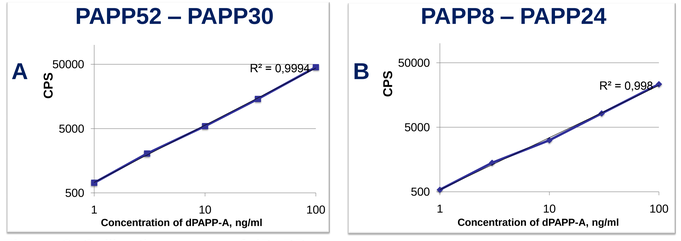


Figure 3. Calibration curves of dPAPP-A assays
Detection limit is 1 ng/ml for recombinant dPAPP-A.

dPAPP-A levels in plasma samples of 43 ACS patients (acute myocardial infarction, unstable angina, 3-20 hours after onset of the chest pain) and 34 non-ACS patients control group were measured.

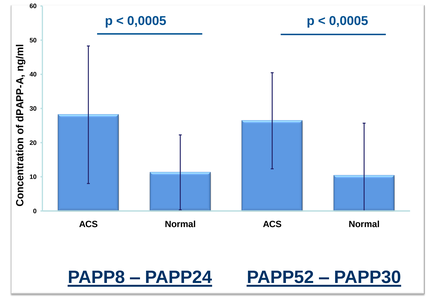


Figure 4. dPAPP-A concentration in plasma samples of 43 ACS patients (acute myocardial infarction, unstable angina) and 34 non-ACS patients control group measured by two assays - **PAPP8 – PAPP24** and **PAPP52 - PAPP30.**

The analysis revealed that dPAPP-A levels in plasma from ACS patients were 2.74 and 2.77-fold higher than in plasma samples of control group, measured by PAPP8-PAPP24 and PAPP52-PAPP30 assays, respectively.

Conclusions

1. **dPAPP-A specific MAbs were obtained and tested with different forms of the antigen. New MAbs have no crossreaction with htPAPP-A**
2. **New type of immunoassay suitable for direct measurement of dPAPP-A in patients' plasma is reported here for the first time**
3. **Significant difference in dPAPP-A level in plasma of ACS patients and control group was shown**

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HyTest