

CLINICAL STUDY

Pregnancy-associated plasma protein A (PAPP-A): theoretical and clinical aspects

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Abstract

Pregnancy-associated plasma protein A (PAPP-A) is an important pregnancy protein. PAPP-A exists in pregnancy serum as a heterotetrameric 2:2 complex with the proform of eosinophil major basic protein (proMBP), forming an approximately 500 kDa and called PAPP-A/proMBP. The gene of PAPP-A has been assigned to human chromosome 9q 33.1. PAPP-A belongs to the metzincin superfamily of metalloproteinases. It contains five short consensus repeats (SCR) and three the lin-notch repeats (LNR) and in addition a putative Zn binding site. The main site of both PAPP-A and proMBP synthesis during pregnancy is the placenta as shown by in situ hybridization. PAPP-A seems to be the predominating IGFBP-4 proteinase in pregnancy serum.

In the women the levels of PAPP-A are highest during pregnancy, when plasma levels increase by a factor of about 150 as compared to the nonpregnant state. PAPP-A is the most abundant in the peripheral maternal circulation. Determination of PAPP-A in pregnancy serum has a limited value in some complication in gravidity such as a threatened abortion, ectopic gravidity, preeclampsia or diabetes mellitus. PAPP-A determination will gain increasing importance since this protein seems to be the major biochemical marker of Down syndrome in the first trimester of pregnancy. Maternal serum levels of PAPP-A in the first trimester are significantly reduced when a fetus affected by Down syndrome is present. Low first trimester maternal serum levels were found not only in trisomy 21 but also in non-Down syndrome fetal aneuploidies. Another contribution of PAPP-A determination may be in differentiation of stable and unstable angina pectoris. (Tab. 3, Fig. 5, Ref. 78.)

Key words: pregnancy-associated plasma protein A, proform of major basic protein, pregnancy, prenatal screening, Down syndrome.

Abbreviations: DS — Down syndrome, IGFBP — insulin-like growth factor binding protein, LNR — lin-notch repeats, MS — maternal serum, MoM — multiple of the median, NT — nuchal translucence, PAPP-A — pregnancy-associated plasma protein A, proMBP — proform of major basic protein, SCR — short consensus repeats.

It has been long known that the human placenta produces a wide variety of “specific” proteins, which do not occur or occur only in trace amounts in normal sera. During pregnancy they appear in the maternal blood stream or their concentration is strongly elevated. In addition to the well-known hormones human chorionic gonadotrophin (HCG) and human placental lactogen (HPL) during last decades other proteins have been added. One of them is pregnancy-associated plasma protein A

(PAPP-A). It was first partially purified together with other pregnancy-associated plasma proteins B, C and D from pregnancy serum by Lin and his co-workers in 1974 (Lin et al, 1974). The preliminary observation on PAPP-A measurements in late pregnancy has led to optimistic conclusions especially in predicting the occurrence of various obstetric abnormalities but later the

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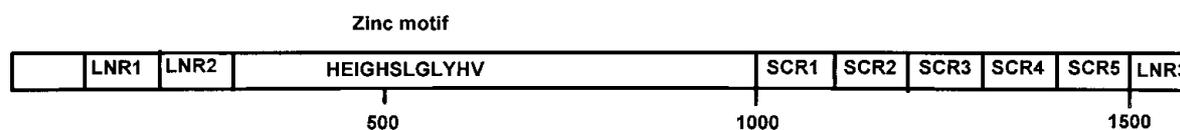


Fig. 1. Schema of the PAPP-A monomer with characteristics motif. SCR – short consensus repeats, LNR – lin-notch repeats

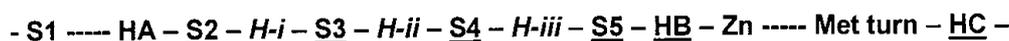


Fig. 2. Predicted secondary structure of the proteolytic domain of PAPP-A (according to Bold et al, 2001). S1—S5 — β -strands, HA, HB, HC, H-i, H-ii, H-iii — α -helices, H-i, H-ii, H-iii — family specific elements, Zn — zinc binding motif, M — Met-turn, elements underlined — common for all members of metzincin superfamily.

interest about PAPP-A has fallen. New period of PAPP-A research has appeared after the publication of Brambati et al (1990), who described decreased levels in the pregnancy with fetus affected by Down syndrome.

Characterization of PAPP-A molecule

Structure of PAPP-A

Earlier studies reported that PAPP-A was a high molecular weight homotetrameric glycoprotein about molecular weight 750-820 kDa with an isoelectric point $pI = 4,4$ (Lin et al, 1974; Bischof et al, 1979). After PAPP-A discovery by Lin et al (1974) in the plasma of pregnant women subsequent purification and characterization demonstrated that PAPP-A was a macromolecular glycoprotein (Mr 800 kDa) of dimeric structure, each monomer being composed of two apparently identical subunits with a molecular weight of 200 kDa.

It has now been demonstrated that PAPP-A exists in pregnancy serum as a heterotetrameric 2 : 2 complex with the proform of eosinophil major basic protein (proMBP), forming an approximately 500 kDa and called PAPP-A/proMBP (Oxvig et al 1993). In non-pregnancy individuals PAPP-A is found as a 400 kDa homodimer (Overgaard et al, 2000).

The PAPP-A gene has been assigned to human chromosome 9q33.1 (Silahtaroglu et al 1993). The aminoacid sequence of PAPP-A has been determined from partial protein sequencing and from sequencing of cloned cDNA (Kristensen et al, 1994).

The cDNA sequence PAPP-A shows that the serum form is derived from a preproprotein with a putative 22-residue signal peptide and a pro part of 58 residue. The PAPP-A mature polypeptide contains 1547 aminoacid residues and 14 putative N-glycosylation sites which are probably all occupied. Seven Ser residues for putative attachment of glycosaminoglycans were described. Some of which are occupied but no galactosamine-based carbohydrate groups are present. PAPP-A subunit contains 82 half-cystine residues which are all bridged. The Cys residues are grouped in several relatively large clusters suggesting structural domains (Bode et al, 1996; Kristensen et al, 1994).

PAPP-A belongs to the *metzincin superfamily of metalloproteinases* (Bode et al, 1993). The superfamily includes four

families of zinc peptidases with members from both the prokaryotes and eucaryotes – actacins, reprotlysins, serralysins and matrix metalloproteinases (MMPs) also called matrixins. PAPP-A may be considered as the first member of a new metzincin family, the *pappalysins* (Tab. 1). The elongated zinc-binding motif is found in the metzincins structure. Besides zinc-binding motif all members share strictly conserved methionin residue as a part of superimposable “Met-turn”, which is supposed to be important for the integrity of the active site. It is known that all metzincin catalytic domains share a common core architecture of five β -strands constituting one β -sheet, and three α -helices. The active site lies along the edge of an outer strand of the β -sheet in a cleft between two half-domains (Stocker et al, 1995).

The molecule of PAPP-A possesses sequence motifs common to metzincin *superfamily of metalloproteinase*, but it is distinct in several regards from the previously classified its four members.

A putative Zn^{2+} binding site, which has been found in PAPP-A molecule, is very similar to that containing the active site Zn^{2+} of the matrix metalloproteinases and quite similar to these of other metalloproteinases (Kristensen et al, 1994). It is interesting that in PAPP-A the linear distance between the zinc motif and the conserved Met residue is 63 aminoacids whereas in other metzincins this distance is shorter – between 7 and 44 residues (Stocker et al, 1995).

In addition to the elongated zinc-binding motif the C-terminal part of PAPP-A contains five approximately 60-residue mo-

Tab. 1. The metzincins superfamily (adapted according to Bold et al, 2001).

Family	Members (examples)
Astacins	Bone morphogenetic protein 1 Cray fish collagenolytic enzymeastacins
Reprotlysins/adamalysins	Snake venom proteinases A disintegrin and metalloproteinase 12
Serralysins	alkaline proteinase bacterial proteinases
Matrix metalloproteinases (MMPs)	human neutrophil collagenase matrilysin
Pappalysins	PAPP-A PAPP-A2

tifs related to *the short consensus repeats (SCR)*. The SCR containing proteins comprise three classes of plasma and membrane proteins. Class I SCR includes complement system. Class II SCR involves the selectins. PAPP-A differs from both of these classes and therefore is defined as class III (Kristensen et al, 1994).

Further molecule of PAPP-A contains three approximately 26-residue motifs related to *the lin-notch repeats (LNR)*. These motifs are similar to the LNRs found in several homeotic gene products regulating early tissue differentiation in their presumed extracellular parts. Schema of the PAPP-A monomer with its characteristics motif is shown in the Figure 1.

The secondary structure of PAPP-A was predicted (Boldt et al, 2001). Secondary structure elements – five β -strands (S1-S5) and three α -helices (HA – HC), that are common to all metzincin, were described for the proteolytic domain of PAPP-A. Besides these elements α -helices H-i, H-ii, H-iii were predicted to be inserted between S2 and S3, S3 and S4 and S4 and S5 respectively. H-ii and H-iii helices have not been observed in known metzincins (Fig. 2).

In vivo PAPP-A is likely to be secreted as an active proteinase following intracellular cleavage. Other regions of the PAPP-A polypeptide, possible on the C-terminal side of the proteolytic domain, could be important for proper folding and secretion (Overgaard et al, 2000).

A comparison of the PAPP-A nucleotide and aminoacid sequences with sequences contained in the Genbank, MIPSX and Swissprot databases did not find any global similarities with known nucleotide or aminoacid sequences (Kristensen et al, 1994). But in 2000 Farr et al (2000) have cloned a novel human cDNA encoding a PAPP-A homologous human protein from placenta cDNA. It was termed pregnancy-associated plasma protein E. The dedicated PAPP-E aminoacid sequence shows global similarity with PAPP-A. Both proteins show about 44 % identical and 62 % similar aminoacids, a corresponding size of 1547 for PAPP-A and 1542 for PAPP-E, a homologous domain structure and a conserved pattern of cysteine residues. According to the PAPP-A motifs the PAPP-E sequence comprises a putative prepropeptide, Met-turn and a putative zinc-binding site, five motifs that are related to the lin-notch motif. The PAPP-E gene

has been assigned to chromosome 1 and is predominantly expressed in placenta. The biological role of PAPP-E remains to be analysed (Farr et al, 2000). Proteins possessing significant partial homologies with PAPP-E are P-, E-, and L-selectins (CD62), complement receptor types 1 and 2, complement factor H and a catalytic domains of metzincins (Farr et al, 2000). Independently on Farr et al (2000) the other work group of Overgaard et al (2001) has described the same protein denoted as PAPP-A2. They showed that PAPP-A2 specifically cleaved IGFBP-5 at one site. Therefore PAPP-A2 is a likely candidate to IGFBP-5 proteinase. With PAPP-A PAPP-A2 defines the new, fifth family of the metzincin superfamily of metalloproteinase, the pappalysins (Boldt et al, 2001; Overgaard et al, 2001).

Complex PAPP-A/proMBP

Circulating PAPP-A is a disulfide bridged complex with proform of eosinophil major basic protein (proMBP) (Oxvig et al, 1993) (Tab. 2).

In nonreducing SDS/PAGE, PAPP-A/proMBP shows a molecular mass of \gg 500 kDa (Oxvig, 1993). The subunit of the PAPP-A/proMBP complex can be irreversibly separated by reduction of disulphide bonds and denaturation. In reducing SDS-PAGE, the PAPP-A subunit has an apparent molecular mass of 200 kDa (Oxvig et al, 1993).

A constituent of circulating PAPP-A is MBP derived from 222-residue preproMBP, containing a presumed 15- or 16-residue signal peptide. Proform of MBP composed of 207 residue is processed to generate mature MBP of 117 residues. The propeptide is acidic (pI = 4,0), extensively and very heterogeneously glycosylated, on the other hand MBP is highly basic (pI = 11) and nonglycosylated (Barker et al 1988; Popken-Harris et al, 1998). ProMBP is less toxic than mature MBP, which is cytotoxic to mammalian cells. It plays multiple roles in the effector function of these cells and may cause the tissue damage associated with eosinophil infiltrates. MBP is the most abundant component of the specific granule of the eosinophil leukocyte. It constitutes more than 50 % of the protein content of the granules in eosinophil leukocytes, from which released by degranulation. Proform MBP is also synthesized by the placenta

Tab. 2. Protein and genetic parameters of PAPP-A and proMBP (according Barker et al, 1988; Oxvig et al, 1994).

Parameter	PAPP-A	proMBP
Number of aminoacids of polypeptide	1547	207
	mature polypeptide	
Size of carbohydrate	26.5 kDa	14.8 kDa
	13.4 %	38.6 %
Molecular mass of PAPP-A monomer	\gg 200 kDa	38 kDa
Molecular mass of complex PAPP-A/proMBP	\gg 500 kDa	
pI	5.4	6.2
Chromosomal localization	9	11
Main source in the pregnancy	syncytiotrophoblast placental X cells	placental X cells

and secreted into the maternal circulation (Popken-Harris et al, 1994, 1998).

Pregnancy serum contained a molar excess of proMBP compared with PAPP-A (Oxvig et al, 1995). ProMBP is complexed not only with PAPP-A but also with angiotensinogen synthesized by the placenta and complement C3 dg, which is a strong immunomodulator (Oxvig et al, 1993, 1995). The association of circulating PAPP-A does realized with proMBP through disulphide bridge formation (Oxvig et al, 1993). Possibly proMBP contain free cysteinyl SH-groups, the mechanism of complex formation probably involves thiol-disulfide exchange engaging one or more particularly exposed PAPP-A disulphide bridges (Oxvig et al, 1993). The covalent PAPP-A/proMBP complex must rise in the extracellular compartment after secretion, because in the placenta, PAPP-A and proMBP are not synthesized in the same cell types (see below). Overgaard et al (2000) assumed that complex formation requires a specific interaction between the two proteins. But there is not necessary for proMBP to interact specifically with the active site of PAPP-A, as long as IGFBP-4 cannot reach this due to steric hindrance or due to a proMBP-induced allosteric change in the conformation of PAPP-A. A model based on steric hindrance is preferred because disulphide linkage between cysteine residues in proMBP and PAPP-A, close to the active site in the primary structure, had been demonstrated. A specific interaction between one cysteine residue of proMBP and the active site zinc atom of PAPP-A must be also considered as a possible inhibitory mechanism. This may be similar as the propeptide cysteine switch mechanism known from other metzincins (Overgaard et al, 2000).

Synthesis PAPP-A

The main site of both PAPP-A and proMBP synthesis during pregnancy is the placenta as shown by in situ hybridization. Both PAPP-A and proMBP belong among the most highly expressed genes in placenta. PAPP-A mRNA have been localized to the syncytiotrophoblast and trophoblast-derived septal X cells, whereas proMBP mRNA has been localized to the placental X cells only (Bonno et al, 1994 a, b; Wagner et al, 1994). The ratio between the specific abundance of proMBP and PAPP-A mRNA in placenta changes during pregnancy: levels of both mRNA species are lower in first trimester placenta than in term placenta, the levels of PAPP-A mRNA increase relatively more than the level of proMBP (Overgaard et al, 1999). This finding is in good agreement with the value in the molar ratio of proMBP and PAPP-A serum levels. It goes from 10-fold excess of proMBP in the first trimester to a 4-fold excess in the third trimester (Oxvig et al, 1995).

Analyses by reverse transcriptase-polymerase chain reaction revealed that both PAPP-A and proMBP mRNA are present in several reproductive and nonreproductive tissue although the levels are much lower than in the placenta. The low mRNA amounts of nonplacental tissue are reflected in the very low concentration of PAPP-A and proMBP proteins in serum of non-pregnant women and men (Qin et al, 1997; Overgaard et al, 1999) reported that PAPP-A and proMBP mRNA is synthesized by fe-

male reproductive tissue, i.e. ovary, tuba uterina, endometrium and myometrium from postmenopausal women in addition to placenta. Synthesis of both species also occurs in nonreproductive tissue, i.e. kidney, colon, bone marrow cells, breast and breast carcinoma. PAPP-A secretion has also been demonstrated from osteoblasts and marrow stromal cells, from granulosa cells and from vascular smooth muscle cells, all of which have known IGF-dependent IGFBP-4 proteinase activity (Overgaard et al, 1999).

Immunohistochemical examination based on techniques using polyclonal antibodies which is less specific reveals quite different types of PAPP-A reactive cells. PAPP-A positivity comprises three different types of specialized cells of mesenchymal origin: endocrine cells, hepatocytes, and hematopoietic cells. Positive epithelial cells are found in the digestive as in the genitourinary tract. The Leydig cell shows the strongest and most consistent immunohistochemical reactivity. In the extragenital organs, a wide variety of PAPP-A reactive cells exists. All these cells have in common their increased frequency in immature rather than in mature cells, in fetal rather than adult organs, in proliferative rather than secretory (Schindler and Bischof, 1984).

Function of PAPP-A

Knowledge of PAPP-A biological function has been lacking from its discovery in 1974 till 1999.

In past several functions have been attributed to PAPP-A such as zinc carrier proteins and barrier against phagocytic-proteolytic defence. Inhibitory effects of leukocyte elastase and lectin induced lymphoblastogenesis, have been also described (Martin-du-Pan et al, 1983; Sinosich et al, 1983 a, 1984).

PAPP-A seems to be *the predominating insulin-like growth factor binding protein (IGFBP) proteinase in pregnancy serum*. In 1999 Lawrence with his co-workers (Lawrence et al, 1999) reported the isolation of an IGF-dependent IGFBP-4-specific protease from human fibroblast and its identification as pregnancy-associated plasma protein A. Thus the IGF-dependent IGFBP-4 protease/PAPP-A is a new member of the metzincin family of metalloproteases involved in normal and pathological insulin-like growth factor (IGF) physiology. PAPP-A represents the predominant IGFBP-4 protease in pregnancy serum and specifically cleaves IGF-binding protein 4 (IGFBP-4) which results in release of IGF bound to IGFBP-4. By degrading IGFBP-4 PAPP-A enhances the bioactivity of IGF. This enzyme is unique among other multifunctional proteases capable of degrading IGFBP-4. Besides IGFBP-4 PAPP-A may in part contribute to IGFBP-5, but not IGFBP-3 proteolytic activity in pregnancy serum (Byun et al, 2001; Overgaard et al, 2000).

The function of PAPP-A must be judged in connection with proMBP. A comparison between rPAPP-A and pregnancy serum PAPP-A/proMBP complex surprisingly demonstrates a difference greater than 100-fold in proteolytic activity. It shows that proMBP functions as a proteinase inhibitor in vivo. It was shown that pregnancy serum and plasma contains trace amount (<1 %) of uncomplexed PAPP-A with a much higher specific activity than PAPP-A/proMBP complex. The measurable activity of the PAPP-

-A/proMBP complexes is probably done by the presence of partly inhibited PAPP-A that exists in a 2 : 1 complexes with proMBP. The finding that proMBP inhibits PAPP-A in pregnancy serum may explain a biological function of this protein outside the eosinophil leukocyte. It also represents a novel mechanism of proteinase inhibition characterized by the enzyme covalently bound by disulphide bonds to its inhibitor (Overgaard et al, 2000).

Because of the high PAPP-A concentration in pregnancy serum, proMBP inhibitory function is likely to be important for circulating PAPP-A that uninhibited would cause a dramatic increase in IGFBP-4 proteinase activity in the circulation. An unusually high IGFBP-4 proteinase activity resulting from uncomplexed PAPP-A may be required locally for placental development (Overgaard et al, 2000).

An inhibitory role may also be important not only in pregnancy but also in non-pregnant individuals, although the synthesis of both PAPP-A and proMBP is much lower. It has been suggested that the IGF-dependent IGFBP-4 protease PAPP-A may play an important role in local proliferative responses such as atherosclerotic plaque development, wound healing, bone remodelling likewise some aspects of human reproduction (Lawrence et al, 1999).

The proliferation of certain cells may be stimulated in an autocrine or paracrine manner, by their secretion of PAPP-A that in turn would cause an increased values of bioactive insulin-like growth factor. Neighbouring cells of a different kind that may synthesise and secrete proMBP have been the ability to control this proliferation by inhibiting the enzymatic activity of PAPP-A (Overgaard et al, 2000). It is known that cultured fibroblasts synthesize PAPP-A, and not proMBP, and in the placenta PAPP-A and proMBP are synthesized in different cell types as shown by in situ hybridization (Lawrence et al, 1999).

However, although proMBP is likely to play an inhibitory role for PAPP-A in the circulation, it cannot be excluded that its role may be different elsewhere. It is tempting to speculate that PAPP-A/proMBP represents a latent form of PAPP-A that become active under given circumstances at the cell surface (Overgaard et al, 2000).

PAPP-A in normal non-pregnant adults

Bischof et al (1982) found PAPP-A *plasma concentration* 120.1 ± 30 ng/ml for proliferative phase and 115.1 ± 23.4 ng/ml for secretory phase and 93.2 ± 22.1 ng/ml for postmenopausal women, so that plasma concentrations of PAPP-A are similar in proliferative and secretory phases of the menstrual cycle. On the contrary Sinosich (1988) was not able to demonstrated PAPP-A in the plasma of non-pregnant women.

PAPP-A concentration in inactive (menopausal) or proliferative endometrial homogenates were consistently low, but the secretory endometrium had significant higher PAPP-A concentration (Bischof et al, 1982). This finding is consistent with an endometrial production of PAPP-A. Bischof et al (1982) explained the failure to observe a similar fluctuation in plasma levels according to the different endometrium stages by either an active metabolism of PAPP-A or by major contribution to circu-

lating PAPP-A from extrauterine sites. The immunohistochemical distribution in the endometrium shows that during the proliferative phase, especially in the early proliferative stage, some glandular cells are stained strongly with PAPP-A antiserum while stromal cells are generally negative. During late secretory stages mainly stromal cells are PAPP-A positive (Bischof et al, 1984b).

Follicular fluid contains PAPP-A which is immunologically and physicochemically indistinguishable from pregnancy. PAPP-A has been detected in 95 % of the follicular fluids. In healthy follicles, PAPP-A concentrations increased throughout the follicular phase to peak immediately prior to ovulation and strongly correlated to folliculogenesis whereas PAPP-A concentrations in atretic follicles did not significantly change throughout the menstrual cycle (Sinosich, 1985). Results of Hourvitz et al (2000) provide the evidence that the gene encoding PAPP-A is expressed in ovaries of normal cycling women and show that the gene is expressed almost exclusively in healthy granulosa cells and corpora lutea. There was a low level of PAPP-A mRNA expression in the atretical antral follicles examined. Hourvitz et al (2000) suggested that restricted pattern of PAPP-A expression in normal human ovaries may be a functional marker of the dominant follicle and its product the corpus luteum. PAPP-A should play a role in controlling survival, growth and/or differentiation of the dominant follicle and corpora lutea by inactivating the gonadotropin antagonist, IGFBP-4.

Low PAPP-A concentrations were found in 16.3 % of *peritoneal fluids* (Sinosich, 1988).

Seminal plasma contains PAPP-A immunoreactivity in the same form as in the other sites (Martin-du-Pan et al, 1983; Sinosich, 1988). Seminal PAPP-A levels ranged from below assay detection limit to 814 IU/l without no statistical difference in the concentrations between subfertile, infertile and fertile ejaculates. These results suggested that determination of seminal PAPP-A concentrations was of little apparent use in qualitative semen analysis (Sinosich, 1985).

PAPP-A in pregnancy

In the women the levels are highest during pregnancy, when plasma levels increase by a factor of about 150 as compared to the nonpregnant state (Bischof et al, 1984). PAPP-A is the most abundant in the maternal circulation with a mean vascular content of 250 mg at term (Sinosich, 1985).

In women with singleton pregnancies PAPP-A was first detected in the maternal blood about 28 days post implantation (Sinosich, 1985). Serum PAPP-A concentration increases exponentially with a doubling time of 3–4 days during the first trimester, then the levels continue to rise throughout pregnancy until delivery. It can be seen that the concentration rises up at a lesser gradient to 36 weeks after which the levels increase more steeply right up to term (Smith et al, 1979). Maximum levels were attained at term. Our results of the serum levels at the end of the first trimester and beginning of the second trimester are shown in the Figure 3. The spread of PAPP-A value from subject to subject in normal pregnancy analysed Klopper (1982). He noticed two characteristic features. The first one is the very

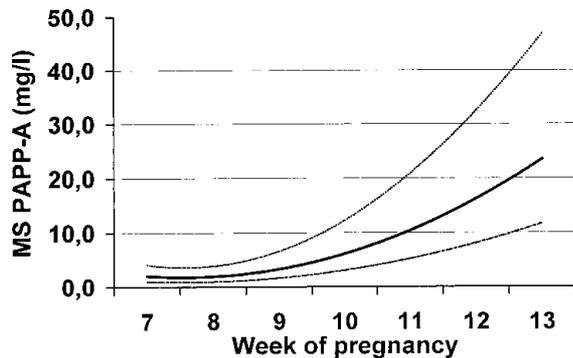


Fig. 3. Maternal serum levels of PAPP-A in 7–13th of pregnancy (Fialová et al, 2001). ____ 1.0 MoM, ___... 0.5 MoM, - - - - 2.0 MoM.

large normal range. It is not a normal Gaussian distribution, but the skew distribution with a few wild high values which distort the upward extension. This is the second characteristic feature.

After parturition the clearance of PAPP-A from peripheral blood is slower than other molecules produced by the trophoblast, the average half life of PAPP-A after normal delivery is 52.9 ± 25.8 hours (Bischof et al, 1984 a). At termination in the first trimester the half life is 51 hours. After surgical termination of ectopic pregnancy in patients with curetted decidua, PAPP-A disappeared significantly faster than in women with intact decidua. Then the half-life of PAPP-A is longer in presence of the decidua than in absence. These results indicate that the production of PAPP-A continues by the decidua after removal of the trophoblast in early pregnancy (Bischof et al, 1984 a).

Only minor pools of PAPP-A are distributed outside the maternal circulation. Trace amount of PAPP-A has been detected in amniotic fluid, colostrum and fetal blood. The concentrations seen in the fetal circulation are 1000 fold less and levels of PAPP-A in amniotic fluid are at least 10-fold less than in the maternal circulation (Duberg et al, 1982). The total PAPP-A mass distribution across the fetoplacental-maternal compartments indicates predominating PAPP-A secretion from the trophoblastic cells into the maternal circulation (Sinosich, 1985).

Westergaard et al (1983 a) found a statistically significant correlation between PAPP-A serum concentration and maternal weight ($r = -0.16$, $p = 0.0004$) and placental weight ($r = 0.175$, $p = 0.0001$) and gravidity ($r = -0.11$; $p = 0.02$). The inverse relationship between PAPP-A levels and maternal body weight is probably explain by the presence of a larger plasma volume in heavier women (Bischof et al, 1980). The mean levels of PAPP-A are higher in primigravidae. The influence of gravidity on PAPP-A levels is interesting but the biological significance of this association is unclear as the placental weights do not differ between primigravidae and multigravidae. The finding of positive correlation between PAPP-A and placental weight is expected observation for proteins produced by trophoblast (Westergaard et al, 1983 a).

It was also described a significant positive correlation between PAPP-A levels and relative birth weight in the first tri-

mester and between maternal serum levels of PAPP-A in late pregnancy and birth weight (Pedersen et al, 1995; Langhoff-Ross et al., 1989). The lower levels of PAPP-A in first trimester predicted poorer fetal growth. It could merely reflect that the fetus in normal gravidity has its given growth potential and tends to stay within borderlines. A lower PAPP-A level in the first trimester may suggest that this fetus is going to become a small but normal baby. On the other hand it could be speculated, that the smaller placenta is responsible for the slower fetal growth, so that there would be a subgroup of normal pregnancies in which the size or capacity of the placenta restricts fetal growth (Pedersen et al, 1995).

Clinical application

Soon after description and isolation of PAPP-A the first clinical studies aimed at its clinical utility as an index of placental function and on its predictive value with respect to pregnancy outcome in threatened abortion, ectopic gravidity and other diseases in pregnancy, have appeared. Since 1990 particular interest in PAPP-A has developed in the connection with the first trimester screening for Down's syndrome.

Spontaneous abortion

Two prospective studies (Westergaard et al, 1983 b, 1985) achieved promising results. In the absence of fetal heart action maternal serum concentration of PAPP-A is depressed. Also if abortion occurs after detection of heart action PAPP-A levels are depressed below the 10th percentile. Abnormal levels were frequently observed weeks before the clinical progress of spontaneous abortion while the fetus was still alive. The authors reported that only abnormal PAPP-A levels in comparison with other pregnancy proteins would distinguish between those pregnancies which are terminated by miscarry from those without embryonic or fetal heart action evident ultrasonically at the time of blood sampling. Ruge et al (1990) reported about 128 women admitted to hospital because of vaginal bleeding in the 7th to 20th gestation week in whose the viability of the fetus was confirmed by ultrasonography. The serum levels were significantly lower in the women with vaginal bleeding than the normally pregnant women. But, with regard to abortion later on, the predictive value an abnormal blood test on admission was only 18.7%. Serial determinations revealed an increase of PAPP-A concentrations corresponding to the centile expected in both group of women who aborted and the group of women who gave birth. Therefore an abnormal test on admission had a low predictive value in the assessment of the prognosis in women with symptoms of threatened abortion.

In the pregnancies after treatment for infertility PAPP-A levels in women who later aborted remained lower (Yovich et al, 1986). Sinosich et al (1983 b) who also studied pregnancies after in-vitro fertilization, found depressed PAPP-A levels in a women who subsequently aborted spontaneously at 17 weeks of gestation, in spite normal ultrasonic finding.

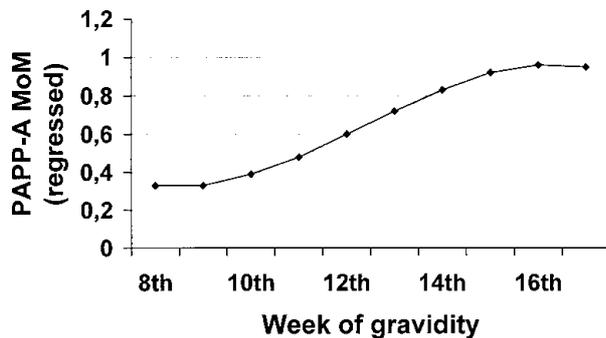


Fig. 4. Maternal serum PAPP-A in regressed MoM in trisomy 21 in the 1st and the 2nd trimester (according data of Cucle and vanLith, 1999).

Ectopic gravidity

PAPP-A levels in blood seem to be depressed in ectopic gravidity (Bischof et al, 1983). In extrauterine gravidity the PAPP-A concentrations were less than the 10th centile of the normal levels for intrauterine pregnancies in 50 % of patients and PAPP-A were not detected in the peripheral circulation in the remainder.

Preeclampsia

Lin et al (1977) showed that levels of PAPP-A were raised in preeclamptic pregnancies. Later studies found that the change was most marked in the more severe grades of preeclampsia and the increase of PAPP-A preceded the advent of hypertension and albuminuria (Klopper, 1982).

Diabetic pregnancy

PAPP-A levels are slightly but not significantly lower in pregnant women with diabetes mellitus despite of its association with large newborns and placental macrosomia (Lin et al, 1977). In the study of Pedersen et al (1998 a, b) significantly depressed PAPP-A levels in diabetic pregnant women in 8th–14th weeks were also observed. Correlation between serum levels of PAPP-A and the severity and duration of diabetes mellitus assessed by the White's classification or with the average blood glucose level as judged from the Hb A_{1c} was not found, but a positive association between relative birth weight and PAPP-A levels in pregnancies complicated by maternal diabetes, a situation in which fetal growth is known to deviate from normal, exists. It was confirmed, that the serum levels of PAPP-A in diabetic mothers are lower in the first trimester so this fact must be taken in account in the interpretation of biochemical prenatal screening results.

Multiple pregnancy

Lin et al (1977) found statistically significant higher levels of PAPP-A in twin pregnancy in the late pregnancy. Westergaard et al (1982) reported that concentrations of PAPP-A in twins in the second half of pregnancy were similar those seen in singleton pregnancy but the majority of measurements (89–97 %) were above the 90th centile. We also confirmed the elevated levels in

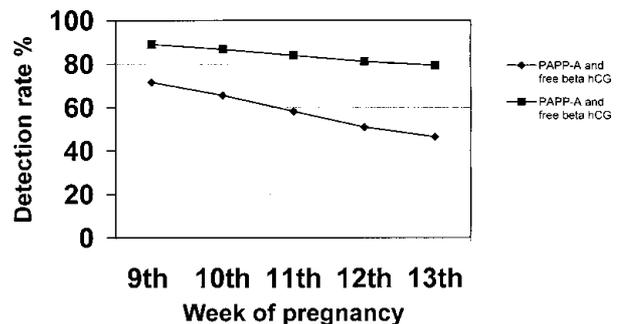


Fig. 5. Estimated trisomy 21 detection rate at 5 % false-positive rate using combination MS PAPP-A and MS free beta HCG and other combination MS PAPP-A, MS free HCG and nuchal translucency (according data of Cucle and vanLith, 1999).

twins in the second trimester of pregnancy (Fialová et al, 2001). For the first trimester, few data are known on the behaviour of PAPP-A in multiple pregnancies. Brambati et al (1997) showed the MoM 1.5, Spencer (2000) found that PAPP-A values were 1.86 times greater than in singletons. Thus the pregnancies with multiple fetuses were frequently associated with increased circulating levels of PAPP-A but a considerable overlap with concentrations in singleton pregnancy exists.

PAPP-A in the pregnancy with congenital abnormalities

1. Trisomy 21

Trisomy 21 (Down syndrome) is the most common chromosomal abnormality in which affected newborn survive beyond infancy. Various analytes have been observed in abnormally high or low levels in serum of pregnant women with fetuses affected by trisomy 21. PAPP-A determination will gain increasing importance since this protein seems to be the major biochemical marker of Down syndrome (DS) in the first trimester of pregnancy. Maternal serum levels of PAPP-A in the first trimester are significantly reduced when a fetus affected by Down syndrome is present. The difference in PAPP-A between trisomy 21 and normal pregnancy decreases with advancing gestation (Fig. 4). Therefore PAPP-A is not available marker for DS in the period of the second trimester (Berry et al, 1997).

From retrospective and case-controlled studies, PAPP-A and free beta-human chorionic gonadotropin have emerged as potential markers of trisomy 21 when measured in maternal serum between 9th to 13th weeks of pregnancy. In 1995 in a multicentric study Wald et al (1995) found out that when PAPP-A and free beta-hCG used together with maternal age should have a detection rate of 62 % at a false-positive rate of 5 %. Krantz et al (1996) reported a 68 % detection efficiency at a 5 % false-positive rates in a case control study of first trimester maternal blood samples from 22 pregnancies with fetuses affected by Down syndrome and 483 control cases. Another prospective study of pregnant women between 9th and 13th weeks' gestation estimated that when PAPP-A and free beta-hCG combined to age 56 % of

the affected cases would have been detected at a false-positive rates of 5 % (Forest et al, 1997). The results of other studies dealing to pregnancy with Down syndrome are shown in Table 3 and Figure 5. In 1999 Canick and Kellner (1999) showed on the basis of 21 published studies with 563 Down syndrome samples that the consensus PAPP-A median in Down syndrome pregnancies was 0.4 MoM.

This is comparable with the results of two-marker second trimester screening of DS. On the basis of Cucle's metaanalysis (Cucle and vanLith, 1999) when ultrasound nuchal translucency (NT) measurement is combined with testing for PAPP-A and free-beta-hCG, the detection rate increases to 86 % and with the four-marker serum profile to 88 %, so that the combination of NT and biochemistry in the first trimester is considerable more sensitive than biochemical screening in the second trimester. With the present availability of new immunochemical methods using time-resolved cryptase emission, it has now become possible to obtain reproducible results of free beta-hCG and PAPP-A within 30 min after collecting blood sample, It is therefore possible to combine the information from ultrasound examination and maternal serum in the same clinical visit. The major advantage includes the ability to deliver the results of prenatal screening quickly, so that women uncertainty anxiety and stress may be reduced (Spencer et al, 1999 b).

2. Trisomy 18

Trisomy 18 belongs to the most common autosomal trisomy after trisomy 21. It is considered a lethal condition with only a small percentage of affected pregnancies continuing to live born infants, who rarely survive beyond the first year of live (Carter et al, 1985).

It seems that PAPP-A in trisomy 18 behaves differently than in trisomy 21. Low diminishing values observed across the first trimester are continued into the second trimester (Bersinger et al, 1999; Spencer, 1999). The levels of PAPP-A MoM are significantly reduced – 0.108. The median PAPP-A MoM in trisomy 18 cases declined with gestational age. At 14–15 weeks the median was 0.185; at 16–17 week was 0.113 and at 18–19 week it was 0.07 (Spencer et al, 1999 a). Of the markers reported to be of value for trisomy 18 in the second trimester PAPP-A is

the most discriminatory. Combination of free beta-hCG and PAPP-A with maternal age would have the ability to detect 74 % of cases at 0.5 % false positive rate or 64 % at 0.1 % false positive rates (Spencer et al, 1999 a).

3. Other chromosomal aneuploidy

Low first trimester maternal serum levels were found not only in trisomy 21 and 18 but also in non-Down syndrome fetal aneuploidies (Ochshorn et al, 2001). Brizot et al (1994) described low median PAPP-A in trisomy 13 with 0.25 MoM and sex chromosome aneuploidies with MoM 0.72. Recently Spencer et al (2000) reported for PAPP-A in trisomy 13 similar results (0.248 MoM). By combination maternal serum levels of PAPP-A and free beta-hCG, NT measurement together with maternal age in a multivariate algorithm, 90 % of cases of trisomy could be detected at a 0.5 % false-positive rate. It was suggested that specific risk algorithms for trisomy 13 should be involved for the first trimester screening similarly to those for trisomy 21 (Spencer et al, 2000).

Bersinger et al (1995) suggested that in DS and other fetal trisomies fetal as well as placental biosynthetic activities are initially reduced but the placenta which produced HCG, SP1 and PAPP-A “cashes up” in the second trimester while the fetal marker levels e.g. AFP, uE₃ remain lower than in normal pregnancy. The release of placental marker proteins into the maternal blood is probably passive. PAPP-A, characterized by a large molecule, is released slowly and therefore markedly reduced in trisomic early pregnancy. The PAPP-A concentrations in both affected and control groups become similar with advancing gestation and placental compenzation but at a later stage than with HCG and SP1, which are smaller molecules. In trisomy 18, fetus retardation and also poor fetal viability is a common feature and therefore the low PAPP-A in the second trimester may be a feature of impending fetal demise (Spencer et al, 1999 a). Brizot et al (1996) searched gene expression of human pregnancy-associated plasma protein-A in placenta from trisomic pregnancies. Despite of the lower serum levels of PAPP-A no significant differences between controls and pregnancies affected by trisomy 21 and 18 in PAPP-A mRNA expression or PAPP-A concentration in the placental tissues were not found. There was also no significant as-

Tab. 3. PAPP-A in MoM in pregnancies affected by Down syndrome in published studies.

Reference	PAPP-A (MoM)	Number of patients with Down syndrome fetuses	Week of gestation
Wald et al, 1992	0.23	19	9-12
Brambati et al, 1993	0.27	14	6-11
Brambati et al, 1994	0.31	13	8-12
Iles et al, 1993	0.38	25	8-14
Haddow et al, 1998	0.41	48	10-13
Krantz et al, 1996	0.41	22	10-13
Wald et al, 1996	0.41	77	8-14
Brizot et al, 1994	0.50	45	10-13
Berry et al, 1997	0.50	52	7-13
Spencer et al, 1994	0.62	21	7-14

sociation between either placental protein or maternal serum PAPP-A concentrations in the normal or trisomic pregnancies and the level of placental mRNA. Brizot et al (1996) suggest that the decrease in maternal serum PAPP-A in trisomic pregnancies may be caused by alternations in post-translational events such changes in the release mechanism of the protein, impaired protein transport across the placenta or modified serum stability of PAPP-A.

4. Cornelia de Lange Syndrome pregnancies

Placental PAPP-A “knock-out” in humans appears to be associated with Cornelia de Lange syndrome. This syndrome involve incomplete fetal development and subsequent deformities.

In 1983 a deficiency of pregnancy associated plasma protein A in maternal serum samples from a patients who gave birth to a male infant with Cornelia de Lange syndrome was reported (Westergaard et al, 1983 c). By subsequent immunocytochemical determination in the placenta no PAPP-A could be demonstrated in trophoblast tissue (Westergaard et al, 1983 c). These results were ascertained by Aitken et al (1999) in the study of 19 pregnancies which resulted in the birth of a child with the classical Cornelia de Lange syndrome phenotype. The cause of the reduced levels of PAPP-A in this syndrome is unknown. Aitken et al (1999) assumed that the extent of the reduction and range of concentrations suggest that PAPP-A is unlikely to have a specific role in Cornelia de Lange syndrome, but maternal serum PAPP-A measurement in the second trimester may be of value as an adjunct to ultrasonography in the prenatal diagnosis of this syndrome.

Acute coronary syndromes

Another promising area of PAPP-A determination is cardiology. Bayes-Genis et al (2001) hypothesed that PAPP-A might be a marker of acute coronary syndromes. They found PAPP-A abundantly expressed in atherosclerotic plaque cells and extracellular matrix of ruptured and eroded unstable plaques but not in stable plaques. Serum levels of PAPP-A were also significantly elevated in patients with myocardial infarction and unstable angina however not with stable angina. They suggested that serum PAPP-A levels can identify patients in the beginning of the process of plaque instability.

Since 1974 when PAPP-A has been isolated from pregnancy plasma our understanding about this protein has greatly advanced. The cDNA sequence for PAPP-A has been described and our knowledge about its function has expanded. Now it is clear that the main contribution of PAPP-A determination consists in its use as biochemical marker of the prenatal screening of Down syndrome and other chromosomal aneuploidy in the first trimester of gravidity and maybe in differentiation of stable and unstable angina pectoris.

References

- Aitken DA, Ireland M, Berry E, Crossley JA, Macri, JN, Burn J, Connor JM.** Second trimester pregnancy associated plasma protein A levels are reduced in Cornelia de Lange syndrome pregnancies. *Prenat Diagn* 1999; 19: 706–710.
- Barker RL, Gleich GJ, Pease LR.** Acidic precursor revealed in human eosinophil granule major basic protein c(DNA). *J Exp Med* 1988; 168: 1493–1498.
- Bayes-Genis A, Conover ChA, Overgaard MT, Bailey KRE, Christiansen M, Holmes DR, Virmani R, Oxvig C, Schwartz RS.** Pregnancy-associated plasma protein A as a marker of acute coronary syndromes. *New Engl J Med* 2001; 345: 1022–1029.
- Berry E, Aitken DA, Crossley JA, Macri JN, Connor JM.** Screening for Down's syndrome: changes in marker levels and detection rates between first and second trimester. *Brit J Obstet Gynaecol* 1997; 104: 811–817.
- Bersinger A, Zakher A, Huber U, Pescia G, Schneider, H.** A sensitive enzyme immunoassay for pregnancy-associated plasma protein A (PAPP-A): a possible first trimester method of screening for Down syndrome and other trisomies. *Arch Gynecol Obstet* 1995; 256: 186–192.
- Bersinger NA, Leporrier N, Herrou M, Leymarie P.** Maternal serum pregnancy-associated plasma protein A (PAPP-A) but not pregnancy-specific beta-1-glycoprotein (SP1) is a useful second-trimester marker for fetal trisomy 18. *Prenat Diagn* 1999; 19: 537–541.
- Bischof P.** Purification and characterization of pregnancy-associated plasma protein-A (PAPP-A). *Arch Gynecol* 1979; 227: 315–326.
- Bischof P, Hughes G, Klopfer A.** Relationship of obstetric parameters to the concentration of pregnancy-associated plasma protein A. *Amer J Obstet Gynecol* 1980; 138: 494–499.
- Bischof P, Schindler AM, Obradovic D, Weil A, Faigaux, R, Herrmann W, Sizonenko PC.** Endometrial and plasma concentrations of pregnancy-associated plasma protein-A (PAPP-A). *Brit J Obstet Gynaecol* 1982; 89: 701–703.
- Bischof P, Reyes H, Hermann WL, Sizonenko PC.** Circulating levels of pregnancy-associated plasma protein-A (PAPP-A) and human chorionic gonadotrophin (hCG) in intrauterine and extrauterine pregnancies. *Brit J Obstet Gynaecol* 1983; 90: 323–325.
- Bischof P, Amaudruz M, Weil-Franck C, Baeriswyl JP, Weil A, Hermann WL, Sizonenko PC.** The disappearance rate of pregnancy-associated plasma protein-A (PAPP-A) after the end of normal and abnormal pregnancies. *Arch Gynecol* 1984 a; 236: 93–98.
- Bischof P, Schindler AM, Urner F, Mensi N, Herrmann WL, Sizonenko PC.** Pregnancy-associated plasma protein-A (PAPP-A) concentration in uterine fluid and immunohistochemical localization in the endometrium. *Brit J Obstet Gynaecol* 1984 b; 91: 863–869.
- Bode W, Gomis-Ruth FX, Stockler W.** Astacins, serralytins, snake venom and matrix metalloproteinases exhibit zinc-binding environments and topologies and should be grouped into a common family, the „metzins“. *FEBS Lett* 1993; 331: 134–140.
- Bode W, Grams F, Reinemer P, Gomis-Ruth FX, Baumann U, McKay DB, Stockler W.** The metzincin-superfamily of zinc-peptidases. *Adv Exp Med Biol* 1996; 89: 1–11.
- Boldt HB, Overgaard MT, Laursen LS, Weyer K, Sottrup-Jensen L, Oxvig C.** Mutational analysis of the proteolytic domain of pregnancy-associated plasma protein-A (PAPP-A): classification as a metzincin. *Biochem J* 2001; 358: 359–367.

- Bonno M, Oxvig C, Kephart GM, Wagner JM, Kristensen T, Sottrup-Jensen L, Gleich GJ.** Localization of pregnancy-associated plasma protein-A and colocalization of pregnancy-associated plasma protein-A messenger ribonucleic acid and eosinophil granule major basic protein messenger ribonucleic acid in placenta. *Lab Invest* 1994 a; 71: 560—566.
- Bonno M, Kephart GM, Carlson CM, Loegering DA, Vernof KK, Gleich GJ.** Expression of eosinophil-granule major basic protein messenger ribonucleic acid in placental X cells. *Lab Invest* 1994 b; 70: 234—241.
- Brambati B, Lanzani A, Tului L.** Ultrasound and biochemical assessment of first trimester of pregnancy. 181—194. In: Chapman M, Grudzinskas G, Chard T (Eds): *The Embryo: Normal and Abnormal Development and Growth*. London, Springer Verlag 1990.
- Brambati B, Macintosh MCM, Teisner B, Maguiness S, Shrimanker K, Lanzani A, Bonacchi I, Tului L, Chard T, Grudzinskas JG.** Low maternal serum levels of pregnancy associated plasma protein A (PAPP-A) in the first trimester in association with abnormal fetal karyotype. *Brit J Obstet Gynaecol* 1993; 100: 324—326.
- Brambati B, Tului L, Bonacchi I, Shrimanker K, Suzuki Y, Grudzinskas JG.** Serum PAPP-A and free beta hCG are first-trimester screening markers for Down syndrome. *Prenat Diagn* 1994; 14: 1043—1047.
- Brambati B, Macri JN, Tului L, Hallahan TW, Krantz DA, Alberti E.** First trimester fetal aneuploidy screening: maternal serum PAPP-A and free beta hCG. 135—147. In: Grudzinskas G, Warfe RHT (Eds): *Screening for Down syndrome in the first trimester*. London, RCOG Press 1997.
- Brizot ML, Snijders RJM, Bersinger NA, Khun P, Nicolaidis KH.** Maternal serum pregnancy associated plasma protein A and fetal nuchal translucency thickness for the prediction of fetal trisomies in early pregnancy. *Obstet Gynecol* 1994; 84: 918—922.
- Brizot ML, Hyett JA, Mckie AT, Bersinger NA, Rarzaneh F, Nicolaidis KH.** Gene expression of human pregnancy-associated plasma protein-A in placenta from trisomic pregnancies. *Placenta* 1996; 17: 33—36.
- Byun D, Mohan S, Yoo M, Sexton C, Baylink DJ, Qin X.** Pregnancy-associated plasma protein A accounts for the insulin-like growth factor (IGF)-binding protein-4 (IGFBP-4) proteolytic activity in human pregnancy serum and enhances the mitogenic activity of IGF by degrading IGFBP-4 in vitro. *J Clin Endocrinol Metab* 2001; 86: 847—854.
- Canic JA, Kellner LH.** First trimester screening for aneuploidy: serum biochemical markers. *Semin Perinatol* 1999; 23: 359—368.
- Carter PE, Pearn JH, Bell J, Martin N, Anderson NG.** Survival in trisomy 18. *Clin Genet* 1985; 27: 59—61.
- Cuckle HS, van Lith MM.** Appropriate biochemical parameters in first-trimester screening for Down syndrome. *Prenat Diagn* 1999; 19: 505—512.
- Duberg S, Bischof P, Schindler AM, Beguin F, Herrmann W, Sizonenko PL.** Tissue and plasma concentration of pregnancy-associated plasma protein A (PAPP-A): comparison with other fetoplacental products. *Brit J Obstet Gynaecol* 1982; 89: 352—357.
- Farr M, Strübe J, Geppert HG, Kocourek A, Mahne M, Tschesche H.** Pregnancy-associated plasma protein E. *Bioch Biophys Acta* 2000; 1493: 356—362.
- Fialová L, Matouš-Malbohan I, Mikulíková L, Benešová O, Krofta L, Zwinger A.** Pregnancy-associated plasma protein A (PAPP-A) in the first trimester. *Czech Gynaecol* 2001; 66: 280—285.
- Forest JC, Massé J, Moutquin JM.** Screening for Down syndrome during first trimester: a prospective study using free beta-human chorionic gonadotropin and pregnancy-associated plasma protein A. *Clin Biochem* 1997; 30: 333—338.
- Haddow JE, Palomaki GE, Knight GJ, Williams J, Miller WA, Johnson A.** Screening of maternal serum for fetal Down's syndrome in the first trimester. *New Engl J Med* 1998; 338: 955—961.
- Hourvitz A, Widger AER, Filho FLT, Chang RJ, Aashi EY, Erickson GF.** Pregnancy-associated plasma protein-A gene expression in human ovaries is restricted to healthy follicles and corpora lutea. *J Clin Endocrinol Metab* 2000; 85: 4916—4919.
- Iles RK, Sharma K, Wathen NC, Campbell J, Ward H, Muller F, Grudzinskas JG, Chard T.** hCG, free subunit and PAPP-A composition in normal and Down's syndrome pregnancies. In: *Fourth Conference: Endocrinology and Metabolism in Human Reproduction*, pp.32. London: RCOG, 1993.
- Klopper A.** Biological and clinical aspects of PAPP-A. 333—344. In: Grudzinskas JG, Teisner B, Seppala M (Eds): *Pregnancy Proteins*. Academic Press Australia 1982.
- Krantz DA, Larsen JW, Buchanan PD, Macri JN.** First trimester Down syndrome screening: free beta human chorionic gonadotropin and pregnancy associated plasma protein A. *Amer J Obstet Gynecol* 1996; 174: 612—616.
- Kristensen T, Oxvig C, Sand O, Moller NP, Sottrup-Jensen L.** Amino acid sequence of human pregnancy-associated plasma protein-A derived from cloned cDNA. *Biochemistry* 1994; 33: 1592—1598.
- Langhoff-Ross J, Wibell L, Gebre-Medhin M, Lindmark G.** Placental hormones and maternal glucose metabolism. A study of fetal growth in normal pregnancy. *Brit J Obstet Gynaecol* 1989; 96: 320—326.
- Lawrence JB, Oxvig C, Overgaard MT, Sottrup-Jensen L, Gleich GJ, Hays LG, Yates JR, Conover ChA.** The insulin-like growth factor (IGF)-dependent IGF binding protein-4 protease secreted by human fibroblasts is pregnancy-associated plasma protein-A. *Proc Natl Acad Sci USA* 1999; 96: 3149—3153.
- Lin TM, Halbert SP, Kiefer D, Spellacy WN, Gall S.** Characterization of four pregnancy-associated plasma proteins. *Amer J Obstet Gynecol* 1974; 118: 223—236.
- Lin TM, Halbert SP, Spellacy WN, Berne BH.** Plasma concentrations of four pregnancy proteins in complications of pregnancy. *Amer J Obstet Gynecol* 1977; 128: 808—810.
- Martin-du-Pan R, Bischof P, Bourrit B, Lauber K, Girard J, Herrmann W.** Immunosuppressive activity of seminal plasma and pregnancy-associated plasma protein-A (PAPP-A) in men. *Arch Androl* 1983; 10: 185—188.
- Ochshorn Y, Kupferminc MJ, Wolman I, Orr-Urtreger A, Jaffa AJ, Yaron Y.** First trimester PAPP-A in the detection of non-Down syndrome aneuploidy. *Prenat Diagn* 2001; 21: 547—549.
- Overgaard MZ, Oxvig C, Christiansen M, Lawrence JB, Conover ChA, Gleich GJ, Sottrup-Jensen L, Haaning J.** Messenger ribonucleic acid levels of pregnancy-associated plasma protein-A and the proform of eosinophil major basic protein: Expression in human reproductive and nonreproductive tissues. *Biol Reprod* 1999; 61: 1083—1089.

- Overgaard MZ, Haaning J, Boldt HB, Olsen IM, Laursen LS, Christiansen M et al.** Expression of recombinant human pregnancy-associated plasma protein-A and identification of the proform of eosinophil major basic protein as its physiological inhibitor. *J Biol Chem* 2000; 275: 31126—31133.
- Overgaard MZ, Boldt HB, Laursen LS, Sottrup-Jensen L, Conover ChA, Oxvig C.** Pregnancy-associated plasma protein-A2 (PAPP-A2), a novel insulin-like growth factor-binding protein-5 proteinase. *J Biol Chem* 2001; 276: 21849—21853.
- Oxvig C, Sand O, Kristensen T, Gleich GJ, Sottrup-Jensen L.** Circulating human pregnancy-associated plasma protein-A is disulfide-bridged to the proform of eosinophil major basic protein. *J Biol Chem* 1993; 268: 12243—12246.
- Oxvig C, Sand O, Kristensen T, Kristensen L, Sottrup-Jensen L.** Isolation and characterization of circulating complex between human pregnancy-associated plasma protein-A and proform of eosinophil major basic protein. *Biochim Biophys Acta* 1994; 1201: 415—423.
- Oxvig C, Haaning J, Kristensen T, Wagner JM, Rubin I, Gleich GJ, Sottrup-Jensen L.** Identification of angiotensinogen and complement C3dg as novel proteins binding the proform of eosinophil major basic protein in human pregnancy serum and plasma. *J Biol Chem* 1995; 270: 13645—13651.
- Pedersen J.F, Sorensen S, Ruge S.** Human placental lactogen and pregnancy-associated plasma protein A in first trimester and subsequent fetal growth. *Acta Obstet Gynecol* 1995; 74: 505—508.
- Pedersen JF, Sorensen S, Molsted-Pedersen L.** Serum levels of human placental lactogen, pregnancy-associated plasma protein A and endometrial secretory protein 14 in first trimester of diabetic pregnancy. *Acta Obstet Gynecol Scand* 1998; 77: 155—158.
- Popken-Harris P, Thomas L, Oxvig C, Sottrup-Jensen L, Kubo H, Klein JS.** Biochemical properties, activities, and presence in biologic fluids of eosinophil granule major basic protein. *J Allergy Clin Immunol* 1994; 94: 282—189.
- Popken-Harris P, Checkel J, Loegering D, Madden B, Springett M, Kephart G, Gleich GJ.** Regulation and processing of a precursor form of eosinophil granule major basic protein (ProMBP) in differentiating eosinophils. *Blood* 1998; 92: 623—631.
- Ruge S, Pedersen JF, Sorensen S, Lange AP.** Can pregnancy-associated plasma protein A (PAPP-A) predict the outcome of pregnancy in women with threatened abortion and confirmed fetal viability. *Acta Obstet Gynecol Scand* 1990; 69: 589—595.
- Silahtaroglu AN, Tümer Z, Kristensen T, Sottrup-Jensen L, Tommerup N.** Assignment of the human gene for pregnancy-associated plasma protein A (PAPP-A) to 9q33.1 by fluorescence in situ hybridization to mitotic and meiotic chromosomes. *Cytogenet Cell Genet* 1993; 62: 214—216.
- Sinosich MJ, Davey, MW, Teisner B, Grudzinskas JG.** Comparative studies of pregnancy-associated plasma protein-A and alpha-2 macroglobulin using metal chelate chromatography. *Biochem Int* 1983 a; 7: 33—42.
- Sinosich MJ, Smith DH, Grudzinskas JG, Saunders DM, Westergaard JG, Teisner B.** The prediction of pregnancy failure by measurement of pregnancy-associated plasma protein A (PAPP-A) following in vitro fertilization and embryo transfer. *Fertil Steril* 1983 b; 40: 539—541.
- Sinosich MJ, Saunders DM, Grudzinskas JG.** Pregnancy-associated plasma protein-A: a barrier to maternal proteolytic attack. 209—212. In: Harrison RF, Bonnar J, Thompson W (Eds): *In Vitro Fertilization, Embryo Transfer and Early Pregnancy*. MTP Press, Lancaster, 1984.
- Sinosich MJ.** Biological role of pregnancy-associated plasma protein-A in human reproduction. 158—183. In: Bischof P, Klooper A (Eds): *Proteins of the Placenta*. 5th International Congress on Placental Proteins, Annecy 1984. Karger, Basel, 1985.
- Sinosich MJ.** Pregnancy-associated plasma protein-A. Fact, fiction and future. 45—81. In: Chapman M, Grudzinskas G, Chard T (Eds): *Implantation-Biological and clinical Aspects*. Springer Verlag, Berlin—Heidelberg 1988.
- Schindler AM, Bischof P.** Histochemical localization of pregnancy-associated plasma protein A in fetal, infant, and adult organs and comparison between antisera. *Gynecol Obstet Invest* 1984; 18: 88—94.
- Smith R, Bischof P, Hughes G, Klopper A.** Studies on pregnancy-associated plasma protein A in the third trimester of pregnancy. *Brit J Obstet Gynaecol* 1979; 86: 882—887.
- Spencer K, Aitken DA, Crossley JA, McGaw G, Berry E, Anderson R, Connor JM, Macri JN.** First trimester biochemical screening for trisomy 21: the role of free beta hCG, alpha-fetoprotein and pregnancy-associated plasma protein A. *Ann Clin Biochem* 1994; 31: 447—454.
- Spencer K.** Screening for trisomy 21 in twin pregnancies in the first trimester using free beta-hCG and PAPP-A, combined with fetal nuchal translucency thickness. *Prenat Diagn* 2000; 20: 91—95.
- Spencer K, Ong C, Skentou HW, Liao AH, Nicolaides K.** Screening for trisomy 13 by fetal nuchal translucency and maternal serum free beta-hCG and PAPP-A at 10-14 weeks of gestation. *Prenat Diagn* 2000; 20: 411—416.
- Spencer K, Crossley JA, Green K, Worthington DJ, Brownbill K, Aitken DA.** Second trimester levels of pregnancy associated plasma protein-A in cases of trisomy 18. *Prenat Diagn* 1999 a; 19: 1127—1134.
- Spencer K, Soute V, Tul N, Snijders R, Nicolaides KH.** A screening program for trisomy 21 at 10—14 weeks using fetal nuchal translucency, maternal serum free beta-human chorionic gonadotropin and pregnancy-associated plasma protein-A. *Ultrasound Obstet Gynecol* 1999 b; 13: 231—237.
- Stocker W, Grams F, Baumann U, Reinemer P, Gomis-Ruth FX, McKay DB, Bode W.** The metzins-topological and sequential relations between the astacins, adamalysins, serralyins, and matrixins (collagenases) define a superfamily of zinc-peptidase. *Protein Sci* 1995; 4: 823—840.
- Wagner JM, Hustin J, Bonno M, Kephart GM, Gurian KV, Gleich GJ.** Pregnancy-associated major basic protein: deposition of protein and expression of mRNA at the maternal-fetal junction in early and late gestation. *Placenta* 1994; 15: 625—640.
- Wald N, Stone R, Cuckle HS, Grudzinskas JG, Barkai G, Brambati B, Teisner B, Fuhrmann W.** First trimester concentrations of pregnancy associated plasma protein A and placental protein 14 in Down's syndrome. *Brit Med J* 1992; 305: 28.
- Wald NJ, Kennard A, Hackshaw AK.** First trimester serum screening for Down's syndrome. *Prenat Diagn* 1995; 15: 1227—1240.
- Wald NJ, George L, Smith D, Densem JW, Petterson K.** Serum screening for Down's syndrome between 8 and 14 weeks of pregnancy. *Brit J Obstet Gynaecol* 1996; 104: 407—412.
- Westergaard JG, Teisner B.** Pregnancy associated plasma protein A in normal and abnormal late pregnancy. 345—354. In: Grudzinskas JG,

Teisner B, Seppala M (Eds): *Pregnancy Proteins*. Academic Press Australia, 1982.

Westergaard JG, Teisner B, Grudzinskas JG. Serum PAPP-A in normal pregnancy: Relationship to fetal and maternal characteristics. *Arch Gynecol* 1983 a; 233: 211—215.

Westergaard JG, Sinosich MJ, Bugge M, Madsen LT, Teisner B, Grudzinskas JG. Pregnancy-associated plasma protein A in prediction of early pregnancy failure. *Amer J Obstet Gynecol* 1983 b; 145: 67—69.

Westergaard JG, Chemnitz J, Teisner B, Poulsen HK, Ipsen L, Beck B, Grudzinskas JG. Pregnancy-associated plasma protein A: a possib-

le marker in the classification and prenatal diagnosis of Cornelia de Lange syndrome. *Prenat Diagn* 1983 c; 3: 225—23.

Westergaard JG, Teisner B, Sinosich MJ, Madsen LT, Grudzinskas JG. Does ultrasonic examination render biochemical tests obsolete in the prediction of early pregnancy failure. *Brit J Obstet Gynaecol* 1985; 92: 77—83.

Yovich JL, Willcox DL, Grudzinskas JG, Bolton AE. The prognostic value of hCG, PAPP-A, oestradiol and progesterone in early human pregnancy. *Aust NZ J Obstet Gynaecol* 1986; 26: 59—64.

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