Ned Tijdschr Klin Chem Labgeneesk 2007; 32: 195-196

Circulating RNA: early detection of pre-eclampsia and other disorders

C.B.M. OUDEJANS[1], A. VISSER[1], M. van DIJK[1], M.J. OOSTERKAMP[1], J. MULDERS[1], J. van BEZU[1], A. POUTSMA[1], M.A.M. MULDERS[1], E.M.L. SMETS[1], A.T.J.I. GO[2], A.M.A. LACHMEIJER[3], J.M.G. van VUGT[2] and M.A. BLANKENSTEIN[1]

Pre-eclampsia is a pregnancy-specific, vascular disorder with new-onset hypertension and proteinuria. Pre-eclampsia and related pregnancy-associated disorders, i.e. the Hemolysis, Elevated Liver enzymes and Low Platelets (HELLP) syndrome, affect more than 8 million pregnant women per year, being responsible for 60,000 deaths worldwide. An estimated USD 26 billion is spent on healthcare costs. Given this, early identification of patients at risk is urgently needed. As pre-eclampsia is genetically determined, the preferred, most effective strategy to prevent early and late adverse events starts with understanding its genetics. Secondly, although the clinical symptoms of pre-eclampsia are late, maternal and systemic, pre-eclampsia starts with a local dysfunction of fetal, i.e. placental cells during the first trimester. Diagnostically, this poses an enormous challenge, as the assays required should meet two essential criteria. The assay should permit identification of women at risk prior to the occurrence and appearance of clinical symptoms (presymptomatic). Secondly, the assay should be informative about the primary event: placental dysfunction (direct markers). Indeed, various protein biomarkers of placental origin (PlGF, sFlft1, sENG) display changed levels in maternal serum, but still lack discriminative and predictive power in individual patients (1). In contrast, placental RNA analyzed in maternal plasma permits rapid screening of novel biomarkers, including markers (transcription factors, non-coding RNA) and features (epigenetic changes, allele ratios) not accessible by conventional antibody-based assays (2, 3).

Methods

Women (n=2940) with a medical history of pregnancy-induced hypertension were identified in the medical records of 22 hospitals throughout the Netherlands (4). Affected women were recruited having at least one affected sister and having suffered either from pre-eclampsia, HELLP syndrome (Hemolysis, Elevated Liver enzymes, Low Platelets) or eclampsia during their first pregnancies (strict criteria) or from pregnancy induced hypertension only (mild criteria). Of these, DNA was collected from 3 generations (sibpairs, parents, children) of 150 families yielding a total of 332 affected women of whom 233 met the strict criteria with 241 unaffected relatives. In parallel, plasma samples (n=1993) were obtained during first trimester (weeks 9-14) of women attending the prenatal diagnostic unit. Samples were processed and stored to permit isolation of circulating placental RNA.

Results

Following confirmation of genome-wide linkage, we showed by transcription-based prioritization with mutation analysis that the placentally expressed *STOX1* gene on 10q22 is responsible for the familial forms of pre-eclampsia in Dutch females (4). Interestingly, the Y153H variation in the DNA binding domain of the winged helix protein encoded by *STOX1* is subject to a gain-of-function as demonstrated by differential transactivation of at least two effector genes (van Dijk *et al*., submitted). The transactivation potential of the 153H variant allele is three times higher compared to the Y153 wildtype allele. Using a similar approach of combinatorial genetic screening with acknowledgement that the placental genotype determines the maternal phenotype, we identified a locus linked with the HELLP syndrome (Oosterkamp *et al*., submitted). As for pre-eclampsia, this locus appears to involve a placentally-expressed gene.

The presence and detectability of *STOX1* mRNA along with a panel of 278 RNA biomarkers was analyzed in maternal plasma by RT-PCR. By this, 23 markers fullfilled the criteria of being detectable in first trimester plasma with absence in non-pregnant controls.

Conclusions

Pre-eclampsia is a common disease caused by common polymorphisms in multiplicative genes interacting in the early placenta. We predict that, using placental RNA in maternal plasma, quantitative analysis of the alleles expressed by pre-eclampsia susceptibility genes, along with one or more of these 23 endophenotypic markers, will permit presymptomatic diagnosis of pre-eclampsia and related pregnancy-associated disorders in individual patients.

Departments of Clinical Chemistry[1], Obstetrics/Gynaecology[2] and Human Genetics[3], VU University Medical Center, Amsterdam

E-mail: CBM.Oudejans@vumc.nl

Discussion

Analysis of placental RNA in maternal plasma for prenatal diagnosis of pre-eclampsia and other disorders permits the following:

Allelic expression ratios can be analyzed using single nucleotide polymorphisms (SNPs). For this purpose, a powerful modification permitting robust, direct assessment of fetal chromosome dosage in maternal plasma was introduced recently: the RNA-SNP allelic ratio strategy (5). By quantitive comparison of the allelic expression ratios of a placentally-expressed, chromosome 21-encoded gene, *PLAC4*, the expression differences between two (normal) or three chromosome 21 copies (Down syndrome) become clearly detectable in maternal plasma (5). This approach can theoretically be applied to any gene with placental expression with placental RNA present in the plasma of pregnant females and absent in non-pregnant females.

High-throughput automated assays, such as using DNA chip technology, permitting quantitative and qualitative analysis of most, if not all, placental (allelic) transcripts expressed and present in maternal plasma should be developed for this purpose.

References

1. Venkatesha S, Toporsian M, Lam C, Hanai J, Mammoto T, Kim YM, et al. Soluble endoglin contributes to the pathogenesis of preeclampsia. Nat Med 2005, 12: 642-9.
2. Go ATJJ, Visser A, Mulders MAM, Blankenstein MA, Vugt JMG van, Oudejans CBM. Detection of placental transcription factor mRNA in maternal plasma. Clin Chem 2004, 50: 1413-4.
3. Chim SS, Tong YK, Chin RWK, Lau TK, Leung TN, Chan LYS, et al. Detection of placental epigenetic signature of the maspin gene in maternal plasma. Proc Natl Acad Sci USA 2005, 102: 14753-8.
4. Dijk M van, Mulders J, Poutsma A, Könst AA, Lachmeijer AM, Dekker GA, Blankenstein GA, Oudejans CB. Maternal segregation of the Dutch preeclampsia locus at 10q22 with a new member of the winged helix gene family. Nat Genet 2005, 37: 514-9.
5. Lo YM, Tsui NB, Chiu RW, Lau TK, Leung TN, Henung MM, et al. Plasma placental RNA allelic ratio permits noninvasive prenatal chromosomal aneuploidy detection. Nat Med 2007; 13: 218-23.

Ned Tijdschr Klin Chem Labgeneesk 2007; 32: 196-198

Immuunsuppressiva en hun doeleiwit

J. van PELT, F. ROMIJN, H. DEKTER, H. van ROSSUM en N. SMIT

De introductie van immuunsuppressiva heeft een doorbraak gebracht in de behandeling van patiënten bij orgaantransplantaties. Met name de komst van ciclosporine heeft het succespercentage van niertransplantaties enorm vergroot. Tegenwoordig is de overleving van een transplantaat veelal enkele tientallen jaren en is het rejectiepercentage per jaar slechts enkele procenten. Helaas heeft de chronische behandeling met immuunsuppressiva ook veel bijwerkingen waaronder nefrotoxiteit en een vergrote kans op maligniteiten, vooral van de huid. Teneinde een balans te vinden tussen de minimale kans op afstoting en de reductie van bijwerkingen zijn frequente bloedspiegelbepalingen noodzakelijk om de optimale medicijndosering vast te kunnen stellen ('therapeutic drug monitoring') temeer daar er grote individuele verschillen in farmacokinetiek bestaan. Voor de analyse in volbloed zijn door een aantal firma's immunoassays ontwikkeld en meer recent wordt LC-MS toegepast. Na ciclosporine zijn een aantal andere immuunsuppressiva geïntroduceerd zoals tacrolimus, pimecrolimus, everolimus en sirolimus (zie tabel 1).

Centraal Klinisch Chemisch Laboratorium, LUMC, Leiden

Naast orgaantransplantaties worden genoemde middelen in toenemende mate toegepast bij verschillende auto-immuunaandoeningen zoals M. Crohn en zowel systemisch als topicaal bij ernstige huidziekten. Sirolimus en everolimus lijken daarnaast een serieuze toepassing in de oncologie te krijgen.

Zoals gezegd vergt de dosering van immuunsuppressiva veel aandacht. Bloedspiegelbepalingen worden gebruikt als input voor zogenaamde farmacokinetische modellen (PK) waarmee de juiste dosering bere-

Tabel 1. Immuunsuppressiva, het bindende eiwit en het doeleiwit

Medicament	Bindend eiwit	Doeleiwit
Cyclosporin (Neoral, Sandimmune)	Cyclophiline	Calcineurine
Tacrolimus (FK 506, Prograft, Protopic)	FK-BP12	Calcineurine
Pimecrolimus (Elidel)	FK-BP12	Calcineurine
Sirolimus (Rapamycine, Rapamune)	FK-BP12	mTOR
Everolimus (RAD-001)	FK-BP12	mTOR