

concentrations up to 250 $\mu\text{mol/L}$ (mean difference ranging from -1% to 5%).

Biological evidence of myocardial ischemia remains a diagnostic challenge. Detection of IMA appears to be a promising tool for myocardial ischemia detection in patients with no increases in troponin (1, 2). However, before IMA can be routinely used in clinical practice several questions, including its cardiospecificity, upper reference limit, and albumin relationship, must be answered. This work attempted to answer some of these questions. Release of biological markers from skeletal muscle is a major concern for specific myocardial damage detection. Thus, IMA tested under skeletal muscle ischemia conditions is an appropriate model. To date, only Apple et al. (4) have analyzed IMA in such conditions, showing increased basal IMA values in 31% of marathon runners, a drop to baseline values immediately after a marathon, and a return to increased values in 63% of cases 24–48 h thereafter. Skeletal muscle and gastrointestinal ischemia was implicated as a cause of such delayed increases in IMA values. However, this model of extensive, long-duration skeletal muscle ischemia likely does not reproduce the events occurring during myocardial ischemia.

The forearm ischemia test model, in which transient but complete blood flow occlusion is produced and skeletal muscle works under such conditions, produces skeletal muscle ischemia and is more analogous of myocardial ischemia. In the current study, during the forearm ischemia test, a significant decrease in IMA occurred 1–5 min after exercise, with recovery to basal values thereafter. Mean IMA values were always below our laboratory's 95th reference percentile, and only 9% of the analyzed samples were above this limit. However, our 95th reference limit of 101 kilounits/L was 20% higher than the 85 kilounits/L stated by the manufacturer. This is in accordance with the manufacturer's recommendation indicating the need for reference values derived from populations with the same characteristics as patients to be evaluated with the test. However, it should be noted that the small size of our reference population could also have influenced our 95th percentile.

Forearm exercise in ischemic conditions promoted sharp increases in lactate (fivefold) and ammonia (sevenfold). Because these increases occurred simultaneously with IMA decreases, possible interference of both metabolites on the ACB test was assessed. Addition experiments using a serum pool with both lactate and ammonia added produced different results. IMA values remained unchanged throughout increasing concentrations of ammonia. However, as lactate concentrations increased, IMA values decreased. Final lactate concentrations of 3–11 mmol/L reduced the initial IMA value by 7–25%, whereas concentrations of 4 and 5 mmol/L, which can be observed in clinical practice, decreased IMA values by 8% and 9%, respectively. Although an effect of lactate on the ACB test at plasma lactate concentrations within reference values could be negligible, our data suggest that lactate could interfere in the ACB test. In patients with increased lactate

concentrations, decreasing true IMA values might decrease the diagnostic sensitivity. The potential of an interesting finding was the strong negative association between albumin and IMA values. Each 1 g/L change in albumin within the physiologic range of albumin (35–45 g/L) produced an opposite change of 2.6% in IMA values. This could partly explain IMA differences between populations, such as those observed between our reference value and that stated by the manufacturer. However, the contribution of interinstrument differences cannot be ruled out as a reason for such a difference. It could also suggest the need to evaluate IMA values together with those of albumin to avoid possible false-positive or -negative values in individuals with hypo- or hyperalbuminemia.

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DNA Methylation Changes in Sera of Women in Early Pregnancy Are Similar to Those in Advanced Breast Cancer Patients, Hannes M. Müller,^{1†} Lennart Ivarsson,^{1†} Hans Schröcksnadel,¹ Heidi Fiegl,¹ Andreas Widschwendter,¹ Georg Goebel,² Susanne Kilga-Nogler,³ Horst Philadelphly,⁴ Wolfgang Gütter,⁴ Christian Marth,¹ and Martin Widschwendter^{1*} (Departments of ¹Obstetrics and Gynecology, ²Biostatistics and Documentation, and ³Central Blood Transfusion and Immunology, Medical University Innsbruck, Innsbruck, Austria; ⁴Institutes of Laboratory Medicine in Innsbruck and Wörgl, Tirol, Austria; † H.M. Müller and L. Ivarsson contributed equally to this work; * address correspondence to this author at: Department of Obstetrics and Gynecology, Medical University Innsbruck, Anichstrasse 35, A-6020 Innsbruck, Austria; fax 43-512-504-23112, e-mail martin.widschwendter@uibk.ac.at)

In normal human pregnancy, the uterus and its arterial system, including the decidua and the adjacent third of

the myometrium, are invaded by cytotrophoblasts (1, 2), which initiate conversion of the decidual vascular system from a high-pressure/low-flow system to a low-pressure/high-flow system that meets the needs of the fetus and placenta (3). The trophoblastic invasion in humans occurs between weeks 8 and 18 of pregnancy (4). The invasion process into the uterus shows many similarities to the invasion of malignant cells during metastasis in that both types of cells have to pass through a basal membrane (5). One of the most important diseases of pregnant women, preeclampsia, is known to be associated with a failure of complete trophoblastic invasion in the first third of the myometrium (6).

Epigenetic alterations, including changes in DNA methylation status, are among the most common molecular alterations in human neoplasia (7). DNA methylation changes are also involved in mammalian development, starting with a wave of demethylation during cleavage, followed by genome-wide de novo methylation after implantation (8). Recently, Ohgane et al. (9) reported that the differentiation of a trophoblast lineage is associated with DNA methylation and demethylation. In many cases, aberrant methylation of the CpG island genes has been correlated with a loss of gene expression, and it has been proposed that DNA methylation provides an alternative to gene deletion or mutation for the loss of gene function (7). Moreover, it is now widely known that methylated DNA can be detected in various body fluids, including serum and plasma, and that the methylation status of some genes can be used for early detection of, or even risk assessment for, various types of human neoplasia (10).

Cell-free fetal and maternal DNA can be detected in the maternal bloodstream (11) and is reported to be robust and easy to obtain (12). Cell-free fetal DNA in maternal plasma seems to be of importance in noninvasive prenatal diagnosis (13) and in the diagnosis of some pregnancy-associated diseases, such as preeclampsia (14). Recently, Poon et al. (15) reported the first use of differential DNA methylation in maternal plasma to detect fetal DNA. The cellular origin of the increase in total plasma DNA is unclear at present, but Chan et al. (16) speculated that fetal DNA may be released into the plasma by trophoblasts, whereas maternal DNA may be released into the circulation by the decidua.

In this proof-of-principle study we addressed the question of whether the methylation pattern in the serum of pregnant women early in pregnancy of genes known to be involved in the invasion process shows similarities to the methylation pattern in patients with invasive cancers. We also asked whether such an invasion-specific methylation pattern in serum shows differences between women with normal pregnancies and women developing preeclampsia—a disease known to be accompanied by a disturbed invasion process in the first trimester (6). We chose a panel of three genes (*CDH1*, *TIMP-3*, and *PTGS-2*) known to play key roles in the invasion process of tumor cells (17–19), which are often regulated by promoter hyper-

methylation (20–22), or in the invasion process of trophoblast cells (2, 23–25). We also chose one gene (*BLT1*) involved in the regulation of immune response and regulated by promoter hypermethylation (26). In addition, we selected two genes (*APC* and *RASSF1A*) known to be involved in pathways counteracting metastasis that are reported to be methylated in several human neoplasias (27, 28). Recently, we were able to demonstrate that DNA sequences of these six genes are highly methylated in serum of patients with advanced breast cancer and show less methylation in primary breast cancer patients (29).

We analyzed the methylation status of the above-mentioned six genes early in gestation in 32 serum samples from healthy pregnant women with normal pregnancy outcomes and in serum samples from 17 healthy pregnant women who were later diagnosed with either severe preeclampsia (diastolic blood pressure >110 mmHg and 3+ proteinuria), eclampsia, or HELLP syndrome [gestational age at time of sampling, 10–15 weeks (median, 12 weeks) and 6–17 weeks (median, 12 weeks), respectively]. The serum samples were obtained during normal blood drawing for screening during the early gestational weeks. The median patient ages were 32 years (range, 19.7–41.3 years) for pregnant women with normal pregnancy outcomes and 28.5 years (range, 8.6–42.4 years) for women who later developed severe preeclampsia, eclampsia, or HELLP syndrome. Clinical data for all included patients were analyzed anonymously, and persons performing methylation analyses were totally blinded to the clinical data.

Genomic DNA from serum samples was isolated by use of the High Pure Viral Nucleic Acid Kit (Roche Diagnostics) according to the manufacturer's protocol with some modifications for multiple loading of the DNA extraction columns to obtain a sufficient amount of DNA (29). Sodium bisulfite conversion of genomic DNA and the MethyLight assay for methylation analysis were performed as described previously (30, 31). Primers and probes for the analyzed genes were also as published previously (29). Using the MethyLight assay, we obtained percentages of fully methylated reference values, and in this study we deemed a percentage of fully methylated reference value >0 as positive for methylation.

We detected various degrees of DNA methylation of the six loci in sera from pregnant women early in pregnancy who showed normal as well as pathologic pregnancy outcomes (Table 1). We found statistically significant differences between the methylation status of *APC* in sera of women who later developed severe preeclampsia, eclampsia, or HELLP syndrome ($n = 17$) vs healthy pregnant women ($n = 32$; $P = 0.041$) and an inverse trend ($P = 0.067$) for *RASSF1A* methylation in sera of these two groups of pregnant women (Table 1).

We also compared the methylation profiles of the pregnant women in early pregnancy who had normal as well as pathologic pregnancy outcomes with the methylation status of sera from 10 healthy controls, pretreatment

Table 1. Percentage of samples positive for methylation at a specific gene locus in sera of pregnant women, healthy controls, and breast cancer patients.

Group	Percentage of positive samples at each locus					
	<i>TIMP-3</i>	<i>CDH1</i>	<i>PTGS2</i>	<i>BLT1</i>	<i>APC</i>	<i>RASSF1A</i>
Healthy pregnant women (n = 32)	6	69	88	100	18	56
Pregnant women who later developed preeclampsia, eclampsia, or HELLP (n = 17)	18	59	100	100	47	29
Healthy controls (n = 10)	0	25	30	60	0	10
Patients with primary breast cancer (n = 26)	0	20	39	85	23	23
Patients with advanced breast cancer (n = 10)	20	90	100	100	80	80

sera from 26 patients with primary breast cancer, and sera from 10 patients with metastasized breast cancer. The sera of these patients had been analyzed previously for another reason in a recently published study (29). All 10 control patients underwent core biopsies of the breast and were confirmed to have benign disease of the breast (age range, 20.5–71.5 years; median, 42.4 years). Within the group of primary breast cancer patients (age range, 36.1–83.9 years; median, 58 years), 2, 18, and 6 patients had pT1, pT2, and pT3 cancers, respectively, and 15, 10, and 1 patients had lymph node-negative, -positive, and unknown disease, respectively. The 10 patients with advanced breast cancer were diagnosed with metastases in the bone, lung, brain, or liver (age range, 49.3–68.7 years; median, 53.6 years).

We first addressed the question whether sera from the two groups of pregnant women had a methylation status different from that of healthy controls. We found strong statistically significant differences between the methylation status of *PTGS2* and *BLT1* in sera from pregnant women (n = 59) vs healthy controls (n = 10; $P < 0.001$ for both), a statistically significant difference for *RASSF1A* methylation ($P = 0.038$), a trend ($P = 0.051$) for *CDH1* methylation status, and no significant differences in the *TIMP-3* and *APC* methylation status (Table 1). Looking at the methylation status of these six gene loci in sera from healthy controls vs pretreatment sera from primary breast cancer patients we found no statistically significant differences (data not shown). Otherwise, looking at various methylation changes in healthy controls compared with those in patients with advanced breast cancer revealed statistically significant differences for *CDH1*, *PTGS2*, *APC*, and *RASSF1A* methylation ($P = 0.013$, 0.003, 0.001, and 0.005, respectively), a trend for *BLT1* methylation ($P = 0.087$), and no significant differences for *TIMP-3* methylation (Table 1).

With this proof-of-principle study we show for the first time that methylation changes in *TIMP-3*, *CDH1*, *PTGS2*, *BLT1*, *APC*, and *RASSF1A* can be detected in pregnant women. From our point of view, the most important finding is the similarity between pregnant women and metastasized breast cancer patients in the methylation changes in genes that are known to be involved in metastasis and tumor cell invasion (17–19, 27, 28) or even in the invasion process of trophoblast cells (2, 23–25) and in the regulation of the immune response (26). As sera of

those cancer patients without evidence of metastasis at the time of diagnosis lacked the methylation changes found in advanced breast cancer and pregnancy (Table 1), we speculate that the observed methylation pattern reflects DNA release from invasive cells, specifically trophoblast cells and tumor cells.

In summary, a statistically significant difference in methylation of *APC* was seen in sera of healthy pregnant women and women who later developed severe preeclampsia, eclampsia, or HELLP syndrome, perhaps offering a possible tool for early detection of this severe disease in pregnancy. We also describe for the first time in a phenomenologic way that methylation changes in sera of women in early pregnancy are similar to those in sera of patients with advanced breast cancer. Further studies are needed to clarify the importance of DNA methylation in regulating the invasion process of cells in general and of trophoblast cells in particular.

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Standardized Evaluation of Instruments for Self-Monitoring of Blood Glucose by Patients and a Technologist, Gunn B.B. Kristensen,^{1*} Kari Nerhus,¹ Geir Thue,¹ and Sverre Sandberg^{1,2} (¹ NOKLUS, Norwegian Center for Quality Improvement of Primary Care Laboratories, Division of General Practice, Department of Public Health and Primary Health Care, University of Bergen, Ulriksdal 8c, N-5009, Norway; ² Laboratory of Clinical Biochemistry, Haukeland University Hospital, Bergen, Norway; * author for correspondence: fax 47-55586710, e-mail gunn.kristensen@isf.uib.no)

Self-monitoring of blood glucose (SMBG) is recommended to improve metabolic control for patients with diabetes because tight glycemic control can decrease microvascular complications in individuals with type 1 and type 2 diabetes (1, 2). The worldwide market for SMBG is \$2.7 billion per year, with annual growth estimated to be 10–12% (3). The performance of these instruments is therefore an important issue.

In most studies evaluating instruments for SMBG, the patient is not involved, although user errors account for a large portion of the total error in SMBG (4). In International Organization for Standardization document ISO/FDIS 15197, it is therefore recommended that a user performance evaluation should be performed in addition to testing by a medical laboratory technologist (MLT) (5). We have developed a procedure based on these recommendations in which instruments are tested simultaneously by an experienced technologist and a group of patients under the same conditions, using blood from the same patients. The procedure was evaluated with two instruments: Glucometer Dex from Bayer (1999) and GlucoMen Glyco from Menarini (2002).

Norwegian patients with diabetes (type 1 and type 2) participated. For each meter, 100 patients were randomly divided into two groups, as shown in Supplement 1 of the Data Supplement that accompanies the online version of this Technical Brief at <http://www.clinchem.org/content/vol50/issue6/>. Before the evaluation, one group was given specific training by a MLT (1–2 h) on how to use the new meter, in addition to standard written instructions, whereas the other group received the meter by mail with the same standard instructions. Three lots of strips were used in the test. After receiving their new meter, the user manual, and 50 strips, the patients familiarized themselves with the meter for 2 weeks. In the period between 2 and 4 weeks, the patients performed five glucose measurements in duplicate on themselves on 5 different days. After 4 weeks, the patients met individually with the MLT for a consultation. During the consultation, the patients first performed two measurements on themselves with no instructions from the MLT. Within 5 min, the MLT took a sample from a different finger. With this blood sample, glucose measurements were performed twice on two different meters and twice with the reference method. Because the meters had no underfill detection, the MLT visually checked the strips and marked all