precision has been stated to be 11.6 μmol/L *(10)*. The imprecision (CV) for total bilirubin measurements on the Olympus analyzer ranged from 4.2% at a concentration of 123.1 μmol/L to 2.8% at a concentration of 372.8 μmol/L.

The ability to measure bilirubin concentrations in newborns simply, rapidly, accurately, cost-effectively, and with minimal risk or discomfort has taken on increased importance in the current environment of managed care, capitation, litigation, and brief postpartum hospitalization. Measurement of bilirubin by various transcutaneous techniques has been reported from several studies with mixed results *(5, 7–10, 15–19)*. Race, as assessed by skin color score, did not have an effect on the performance of the BiliCheck method. In addition, other potential confounding factors, such as birth weight, gestational age, and postnatal age, did not affect the BiliCheck device.

The results of our study may have been influenced by several factors. Freezing and storage of samples for HPLC analysis might have led to some degradation of bilirubin. However, the data shown in the Bland–Altman difference plots suggest that bilirubin degradation was not a significant factor. Other factors that might have influenced our results were the demographics of newborns evaluated in our study. All infants were >32 weeks of gestational age, none had undergone phototherapy, and all were <4 days of age. In addition, all transcutaneous measurements were performed in duplicate by a single individual, whereas singleton determinations by multiple caregivers would most likely be performed in routine practice.

Currently, measurement of total bilirubin in serum or plasma of newborns is the standard of care for the assessment of neonatal jaundice. Our results indicate that the transcutaneous measurement of bilirubin is as accurate as bilirubin measured in plasma in a hospital laboratory when HPLC is used as the comparison method. In conclusion, measurement of bilirubin by the BiliCheck device offers an accurate, rapid, and noninvasive means of assessing plasma bilirubin concentrations in neonates.

We thank Respironics (Marietta, GA) for supplying the reagents used in this study. We also acknowledge the cooperation of the newborn nursing staff at Pitt County Memorial Hospital.

References

1. Harish R, Sharma DB. Transcutaneous bilirubinometry in neonates: evaluation of Minolta Air shields jaundicemeter. Indian Pediatr 1998;35:264–7.
2. Maisels MJ, Kring E. Transcutaneous bilirubinometry decreases the need for serum bilirubin measurements and saves money. Pediatrics 1997;99:599–601.
3. Donzelli G, Pratesi S. Transcutaneous bilirubinometry in healthy preterm newborns. Clin Biochem 2000;33:505–8.
4. Dai J, Parry DM, Krahn J. Transcutaneous bilirubinometry: its role in the assessment of neonatal jaundice. Clin Biochem 1997;30:1–9.
5. Rubaltelli FF, Gourley GR, Loskamp N, Modi N, Roth-Kleiner M, Sender A, et al. Transcutaneous bilirubin measurement: a multicenter evaluation of a new device. Pediatrics 2001;107:1264–71.
6. Knudsen A, Ebbesen F. Transcutaneous bilirubinometry in neonatal intensive care units. Arch Dis Child Fetal Neonatal Ed 1996;75:F53–6.
7. Tayaba R, Gribetz D, Gribetz I, Holzman IR. Noninvasive estimation of serum bilirubin. Pediatrics 1998;E28. http://www.pediatrics.org/cgi/content/full/102/3/e28 (Accessed September 2003).
8. Kumar A. Transcutaneous bilirubinometry in the management of bilirubinemia in term neonates. Indian J Med Res 1994;99:227–30.
9. Schumacher RE. Noninvasive measurements of bilirubin in the newborn. Clin Perinatol 1990;17:417–35.
10. Bhutani VK, Gourley GR, Adler S, Kreamer B, Dalin C, Johnson LH. Noninvasive measurement of total serum bilirubin in a multiracial predischarge newborn population to assess the risk of severe hyperbilirubinemia. Pediatrics 2000;E17. http www.pediatrics.org/cgi/content/full/106/2/e17 (Accessed September 2003).
11. Onks D, Silverman L, Robertson A. Effect of melanin, oxyhemoglobin and bilirubin on transcutaneous bilirubinometry. Acta Pediatr 1993;82:19–21.
12. Maisels MJ, Newman TB. Jaundice in full-term and near-term babies who leave the hospital within 36 hours: the pediatrician's nemesis. Clin Perinatol 1998;25:295–302.
13. Gourley GR, Kreamer B, Arend R. Excremental studies in human neonates. Identification of zinc coproprophyrin as a marker for meconium. Gastroenterology 1990;99:1705–9.
14. Bland JW, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurements. Lancet 1986;1:307–10.
15. Tan KL. Transcutaneous bilirubinometry in fullterm Chinese and Malay infants. Acta Paediatr Scand 1982;71:593–6.
16. Knudsen A. Predicting the need for phototherapy in healthy mature neonates using transcutaneous bilirubinometry on the first postnatal day. Biol Neonate 1995;68:398–403.
17. Knudsen A. Prediction and non-invasive assessment of neonatal jaundice in the term healthy newborn infant. Acta Paediatr 1996;85:393–7.
18. Yamauchi Y, Yamanouchi I. Transcutaneous bilirubinometry: serum bilirubin measurement using transcutaneous bilirubinometer (TcB). A preliminary study. Biol Neonate 1989;56:257–62.
19. Robertson A, Kazmierczak S, Vos P. Improved transcutaneous bilirubinometry: comparison of SpectR(x) BiliCheck and Minolta Jaundice Meter JM-102 for estimating total serum bilirubin in a normal newborn population. J Perinatol 2002;22:12–4.

DOI: 10.1373/clinchem.2003.027326

Serum S100B in Pregnancy-Related Hypertensive Disorders: A Case–Control Study, *Adriana P. Schmidt,[1] Adriano B.L. Tort,[2] Olavo B. Amaral,[2] André P. Schmidt,[2] Roger Walz,[3] Janete Vettorazzi-Stuckzynski,[1] Sérgio H. Martins-Costa,[1] José Geraldo L. Ramos,[1] Diogo O. Souza,[2] and Luis V.C. Portela[2*]* ([1] Departamento de Ginecologia e Obstetrícia, Hospital de Clínicas de Porto Alegre, Porto Alegre, RS, Brazil; [2] Departamento de Bioquímica, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil; [3] Centro de Cirurgia de Epilepsia, Hospital de Clínicas, Departamento de Neurologia, Psiquiatria e Psicologia Médica, Universidade de São Paulo, SP, Brazil; * address correspondence to this author at: Departamento de Bioquímica, ICBS, Universidade Federal do Rio Grande do Sul, Avenida Ramiro Barcelos, 2600-Anexo, CEP 90035-003, Porto Alegre, RS, Brazil; fax 55-51-33165540 or 55-51-33165535, e-mail roska@ufrgs.br)

Eclampsia is defined as the occurrence of seizures and/or coma resulting from hypertensive encephalopathy on a background of preeclampsia *(1)*. Eclampsia appears to be caused by a failure of the brain's autoregulatory response to increases in blood pressure, leading to an increase in cerebral perfusion pressure with overperfusion injury similar to that observed in hypertensive encephalopathy *(2, 3)*. Brain edema and hemorrhage ensue, as observed in imaging studies *(4, 5)*, and there is evidence to suggest that these alterations can cause ischemia to brain cells.

These events lead to neurologic symptoms *(6)*, including seizures as well as cortical blindness, aphasia, limb weakness, psychosis, coma, and cerebrovascular accidents. Studies have analyzed various diagnostic methods, such as transcranial Doppler measurements, as a way to evaluate neurologic involvement in preeclampsia *(3, 7)*. To date, however, there is no reliable laboratory marker to identify patients at risk for eclampsia or its related complications.

S100B is a 21-kDa protein physiologically produced and released primarily by astrocytes in the central nervous system (CNS), where it exerts neurotrophic and gliotrophic actions *(8)*. Because ~95% of S100B is located in the CNS, the results of several studies have suggested that an increase in S100B in blood and cerebrospinal fluid could be a potential marker of neural injury, indicating reactive gliosis, astrocytic death, and/or blood–brain barrier dysfunction. Accordingly, increased S100B concentrations in cerebrospinal fluid and/or blood have been reported in several pathologic conditions causing acute and chronic brain injury, such as head trauma *(9)*, stroke *(10)*, schizophrenia *(11)*, and human T-lymphotropic virus type 1-associated myelopathy *(12)*.

Some studies have also evaluated S100B as a marker of recent seizures. Although S100B has been shown to be increased in both cerebrospinal fluid and brain tissue of patients with temporal lobe epilepsy *(13, 14)*, studies in which serum S100B was measured have failed to show an increase after seizures *(15–17)*. Nevertheless, taking into account that the pathophysiology of seizures in eclampsia involves ischemic damage to cells in the CNS, as well as the fact that increased S100B seems to be an indicator of increased intracranial blood pressure *(18)* and of hypertension-related injury during cardiopulmonary bypass *(19)*, it is feasible that an increase in S100B could be observed in eclampsia, perhaps even before the occurrence of seizures.

In this study, serum S100B was measured in pregnant women with eclampsia, preeclampsia, chronic hypertension, or normal blood pressure to determine whether these concentrations could be an indicator of neurologic involvement in pregnancy-related hypertension. This case–control study was conducted between 2000 and 2002 in the obstetric emergency department of a tertiary hospital in southern Brazil. Approval from the institution's ethics review board was obtained before the study, and all patients provided written informed consent for participation. Patients eligible for the study were pregnant women with a gestational age ≥20 weeks admitted for medical evaluation or labor assistance. Exclusion criteria were present diagnosis or past history of neurologic disease, renal dysfunction, seizures related to other causes, HIV infection, or microangiopathies. Obstetric examination was performed, and a questionnaire including information on current pregnancy features and past obstetric, medical, and family histories was obtained. Blood pressure measurements were performed, and patients were classified into four groups according to previously established criteria on high blood pressure in pregnancy *(1)* as follows: (*a*) normal blood pressure [patients with systolic blood pressure (SBP) <140 mmHg and diastolic blood pressure (DBP) <90 mmHg]; (*b*) preeclampsia (patients with SBP >140 mmHg or DBP >90 mm, evidence of proteinuria, and no history of hypertension outside of pregnancy); (*c*) chronic hypertension (patients with SBP >140 mmHg or DBP >90 mmHg with a history of hypertension outside of pregnancy and no evidence of proteinuria); (*d*) eclampsia (patients fulfilling criteria for preeclampsia with the additional manifestation of seizures). Patients with preeclampsia superposed on chronic hypertension and those with gestational hypertension but no proteinuria or other evidence of preeclampsia were not included in the study.

Blood samples (5 mL of venous blood) were collected at the time of admission in an evacuated anticoagulant-free system and sent to the laboratory for centrifugation. In patients presenting with eclampsia, blood was collected up to 6 h after a seizure. Serum was separated from the clot and stored at −70 °C until analysis. S100B was measured by a commercially available immunoluminometric assay (Sangtec 100®) as described previously *(20)*. Briefly, this is a monoclonal two-site immunoassay that uses an antibody covalently bound to isoluminol as a tracer. All samples were measured in duplicate and were analyzed in the same experiment. The S100B calibration curve was linear up to 20 μg/L, and the CV for duplicates across the entire concentration range for the calibrators and samples remained ≤5%. The detection limit of the assay is 0.02 μg/L.

Data are expressed as mean (SD). Statistical analysis was performed with SPSS, Ver. 10.0 (SPSS). Quantitative variables, including S100B serum concentrations, were compared by ANOVA followed by a Duncan post hoc test. Qualitative variables were compared by Fisher exact test. A P value <0.05 was considered to indicate statistical significance.

Fifty patients were enrolled for the study and classified as described above. The clinical and epidemiologic data for the patients are shown in Table 1. Chronically hypertensive patients were older and had more previous pregnancies than the other groups. Both the SBP and DBP were statistically lower in normotensive patients compared with the other groups. None of the patients allocated to the normotensive, chronic hypertension, or preeclampsia groups developed seizures during their stay at the hospital.

As shown in Fig. 1, there were no significant differences among the serum S100B concentrations of women with chronic hypertension [0.186 (0.12) μg/L], preeclampsia [0.185 (0.14) μg/L]. and normal blood pressure [0.147 (0.07) μg/L]. However, serum S100B was significantly higher in eclampsia patients [0.424 (0.194) μg/L] compared with all other groups (P <0.05).

To our knowledge, this is the first study designed to assess serum S100B in pregnancy-related hypertensive disorders. Our results demonstrate increased concentrations of the protein in pregnant women with eclampsia, but not in those with preeclampsia or chronic hyperten-

Table 1. Clinical features of patients and controls.

	Normotensive	Chronic hypertension	Preeclampsia	Eclampsia
n	16	11	18	10
Age, years				
Mean	21.8	32.4[a]	24.8	19.6
SD	4.8	7.2	6.2	6.9
Race, %				
White	69	82	83	80
Black	25	18	17	20
Others	6	0	0	0
Smoking history, %				
Yes	25	27	22	20
No	75	73	78	80
Family history of preeclampsia, %				
Yes	31	36	33	40
No	69	64	67	60
Previous preeclampsia, %				
Yes	0	0	28[b]	10
No	100	100	72	90
No. of previous pregnancies				
Mean	2.3	4.9[a]	2.0	1.2
SD	1.4	3.5	1.6	0.4
Gestational age, weeks				
Mean	34.6	36.8	34.7	33.5
SD	6.4	1.9	4.1	3.9
SBP/DBP,[c] mmHg				
Mean	109.3/67.9[d]	157.8/98.9	146.2/93.8	160.0/102.2
SD	14.4/9.8	26.4/12.7	14.5/12.6	18.0/18.6

[a] Significantly different from preeclamptic, eclamptic, and normotensive patients ($P < 0.05$).
[b] Significantly different from eclamptic, chronically hypertensive, and normotensive patients ($P < 0.05$).
[c] Mean blood pressures during admission.
[d] Significantly different from chronically hypertensive, preeclamptic, and eclamptic patients ($P < 0.05$).

sion, compared with controls. Several cellular events associated with CNS involvement in eclampsia could account for the present findings. The increased S100B may be secondary to cerebral vascular changes leading to overperfusion, edema, and ischemia, as well as to seizures themselves *(2, 4, 6)*. However, it is unlikely that seizures per se are responsible for the increase in serum S100B because studies on epileptic patients have not found any increases *(15–17)*. Therefore, it is possible that the increases in serum S100B observed in this study could actually precede the occurrence of seizures or other neurologic manifestations in eclampsia.

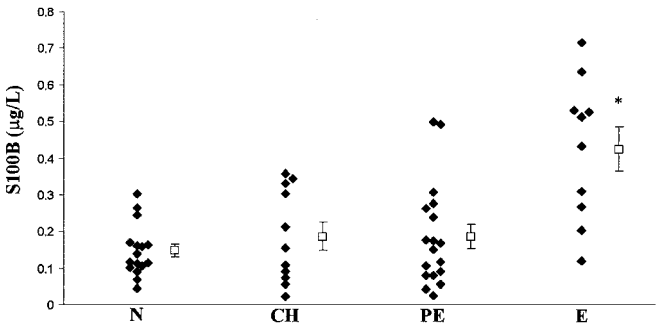

Fig. 1. Scatter plot comparing serum S100B concentrations among four groups of pregnant women.

N, normal blood pressure; *CH*, chronic hypertension; *PE*, preeclampsia; *E*, eclampsia. *, $P < 0.05$ compared with all other groups. *Boxes* and *error bars* indicate means and SD.

Other causes of S100B production that do not involve vascular brain injury cannot be completely excluded by our study. Recent evidence suggests that extracerebral sources may also influence serum S100B *(21)*, although usually in a minor way. Moreover, S100B production by the fetus could also be feasibly playing a role in our findings because concentrations of the protein are known to be quite high in cord blood compared with those found in adult serum *(22, 23)*.

Current diagnosis of imminent eclampsia is based mostly on the clinical assessment of patients with preeclampsia, focusing on neurologic symptoms such as headache and visual alterations. The peripheral measurement of brain proteins such as S100B, however, has been shown to offer a sensitive alternative indicator of cell damage in the CNS when clinical and radiologic assessments are negative. S100B appears to satisfy various criteria for a peripheral marker of brain injuries, such as (*a*) simplicity of measurement with good reproducibility, (*b*) detection in various biological fluids, (*c*) possibility of use in longitudinal monitoring because of its short half-

life, and (*d*) well-established use as an early and quantitative marker of CNS injury. S100B can also offer the additional advantage of providing a quantitative indicator of the extent of brain lesions in various diseases *(10, 11, 13, 14)*, and several previous studies have demonstrated the prognostic value of serum S100B in predicting neurologic prognosis in various conditions, including head trauma, stroke, and cardiac arrest *(9, 10, 18, 24)*.

In spite of the small size of our sample, our findings provide preliminary evidence that increased S100B is associated with eclampsia. Further studies with larger samples are required to determine whether these values can be used to predict the development of eclampsia before it leads to overt clinical manifestations. If this turns out to be the case, the use of S100B measurements to predict CNS involvement in pregnancy-related hypertension might conceivably play a useful role in the clinical management of this condition.

This research was supported by the following Brazilian funding agencies: Conselho Nacional de Pesquisa/Programa Nacional de Excelência (No. 41960904), Fundação de Ampara à Pesquisa do Estado do Rio Grande do Sul; Coordenação de Aperfeiçoamento de Pessoal de Nível Superior; Pro-reitonia de Pesquisa/Universidade Federal do Rio Grande do Sul; and Fundação de Incentivo a Pesquisa e Eventos/Hospital de Clínicas de Porto Alegre.

References

1. Report of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy. Am J Obstet Gynecol 2000; 183:S1–22.
2. Belfort MA, Varner MW, Dizon-Townson DS, Grunewald C, Nisell H. Cerebral perfusion pressure, and not cerebral blood flow, may be the critical determinant of intracranial injury in preeclampsia: a new hypothesis. Am J Obstet Gynecol 2002;187:626–34.
3. Williams K, Galerneau F. Maternal transcranial Doppler in pre-eclampsia and eclampsia. Ultrasound Obstet Gynecol 2003;21:507–13.
4. Naidu K, Moodley J, Corr P, Hoffmann M. Single photon emission and cerebral computerised tomographic scan and transcranial Doppler sonographic findings in eclampsia. Br J Obstet Gynaecol 1997;104:1165–72.
5. Schwartz RB, Feske SK, Polak JF, DeGirolami U, Iaia A, Beckner KM, et al. Preeclampsia-eclampsia: clinical and neuroradiographic correlates and insights into the pathogenesis of hypertensive encephalopathy. Radiology 2000;217:371–6.
6. Rutherford JM, Moody A, Crawshaw S, Rubin PC. Magnetic resonance spectroscopy in pre-eclampsia: evidence of cerebral ischaemia. BJOG 2003;110:416–23.
7. Belfort MA, Anthony J, Saade GR. Prevention of eclampsia. Semin Perinatol 1999;23:65–78.
8. Donato R. S100: a multigenic family of calcium-modulated proteins of the EF-hand type with intracellular and extracellular functional roles. Int J Biochem Cell Biol 2001;33:637–68.
9. Ingebrigtsen T, Waterloo K, Jacobsen EA, Langbakk B, Romner B. Traumatic brain damage in minor head injury: relation of serum S-100 protein measurements to magnetic resonance imaging and neurobehavioral outcome. Neurosurgery 1999;45:468–75.
10. Wunderlich MT, Ebert AD, Kratz T, Goertler M, Jost S, Herrmann M. Early neurobehavioral outcome after stroke is related to release of neurobiochemical markers of brain damage. Stroke 1999;30:1190–5.
11. Lara DR, Gama CS, Belmonte-de-Abreu P, Portela LV, Gonçalves CA, Fonseca M, et al. Increased serum S100B protein in schizophrenia: a study in medication-free patients. J Psychiatr Res 2001;35:11–4.
12. Walz R, Portela LV, Tort AB, Neto EC, Fernandes LN, Gonçalves CA, et al. Serum S100B levels in patients with HTLV-I-associated myelopathy/tropical spastic paraparesis. Neurology 2000;54:2021–2.
13. Griffin WS, Yeralan O, Sheng JG, Boop FA, Mrak RE, Rovnaghi CR, et al. Overexpression of neurotrophic cytokine S100B in human temporal lobe epilepsy. J Neurochem 1995;65:228–33.
14. Steinhoff BF, Tumani H, Otto M, Mursch K, Wiltfang J, Herrendorf G, et al. Cisternal S100 protein and neuron-specific enolase are elevated and site-specific markers in intractable temporal lobe epilepsy. Epilepsy Res 1999;36:75–82.
15. Buttner T, Lack B, Jager M, Wunsche W, Kuhn W, Muller T, et al. Serum levels of neuron-specific enolase and S-100 protein after single tonic-clonic seizures. J Neurol 1999;246:459–61.
16. Palmio J, Peltola J, Vuorinen P, Laine S, Suhonen J, Keränen T. Normal CSF neuron-specific enolase and S-100 protein levels in patients with recent non-complicated tonic-clonic seizures. J Neurol Sci 2001;183:27–31.
17. Leutmezer F, Wagner O, Baumgartner C. Serum S-100 protein is not a suitable seizure marker in temporal lobe epilepsy. Epilepsia 2002;43: 1172–4.
18. Petzold A, Green AJ, Keir G, Fairley S, Kitchen N, Smith M, et al. Role of serum S100B as an early predictor of high intracranial pressure and mortality in brain injury: a pilot study. Crit Care Med 2002;30:2705–10.
19. Schmidt M, Scheunert T, Steinbach G, Schirmer U, Marx T, Freitag N, et al. Hypertension as a risk factor for cerebral injury during cardiopulmonary bypass. Protein S100B and transcranial Doppler findings. Anaesthesia 2001;56:733–8.
20. Portela LV, Tort AB, Schaf DV, Ribeiro L, Nora DB, Walz R, et al. The serum S100B concentration is age dependent. Clin Chem 2002;48:950–2.
21. Missler U, Orlowski N, Notzold A, Dibbelt L, Steinmeier E, Wiesmann M. Early elevation of S-100B protein in blood after cardiac surgery is not a predictor of ischemic cerebral injury. Clin Chim Acta 2002;321:29–33.
22. Gazzolo D, Vinesi P, Marinoni E, Di Iorio R, Marras M, Lituania M, et al. S100B protein concentrations in cord blood: correlations with gestational age in term and preterm deliveries. Clin Chem 2000;46:998–1000.
23. Gazzolo D, Marinoni E, di Iorio R, Lituania M, Bruschettini PL, Michetti F. Circulating S100β protein is increased in intrauterine growth-retarded fetuses. Pediatr Res 2002;51:215–9.
24. Martens P, Raabe A, Johnsson P. Serum S-100 and neuron-specific enolase for prediction of regaining consciousness after global cerebral ischemia. Stroke 1998;29:2363–6.

DOI: 10.1373/clinchem.2003.027391

Determination of Thiopurine Methyltransferase Activity in Isolated Human Erythrocytes Does Not Reflect Putative in Vivo Enzyme Inhibition by Sulfasalazine,

Maria Shipkova,[1] Paul Dieter Niedmann,[2] Victor W. Armstrong,[2] Michael Oellerich,[2] and Eberhard Wieland[1]* ([1] Central Institute for Clinical Chemistry and Laboratory Medicine, Klinikum Stuttgart, Germany; [2] Department of Clinical Chemistry, Georg-August-University Göttingen, Göttingen, Germany; * address correspondence to this author at: Central Institute for Clinical Chemistry and Laboratory Medicine, Klinikum Stuttgart, Katharinenhospital, Kriegsbergstrasse 60, D-70174 Stuttgart, Germany; fax 49-711-2784809, e-mail m.shipkova@klinikum-stuttgart.de)

Determination of thiopurine *S*-methyltransferase (TPMT; EC 2.1.1.67) activity is intended to screen patients before therapy with thiopurine drugs (6-mercaptopurine, 6-thioguanine, and azathioprine) to rule out a deficiency in this enzyme. The enzyme catalyzes the S-methylation of these medicinal agents, a metabolic pathway that competes with the formation of pharmacologically active 6-thioguanine nucleotides (6-TGNs), thereby modulating their therapeutic and toxic effects *(1, 2)*. Individuals with low TPMT activity are known to be at high risk for severe thiopurine-induced myelodepression. In addition to a genetic polymorphism of TPMT alleles, which is responsible for the wide interindividual differences in TPMT activity, cotherapy with various drugs, including aminosalicylates, has been shown to influence enzyme activity