munotech), anti-CD152 (BN13)-PE (Immunotech) and anti-CCR4 (1G1)-PE (Becton Dickinson). For the macrophages analyses, the cells were stained with anti-CD163 (GHI/61)-PE (BD Pharmingen, San Diego, CA, USA), FITC-conjugated anti-CD68 (KIM7) mAb (CALTAG Laboratories, Burlingame CA, USA), anti-CD80 (MAB104)-PE (Immunotech), anti-CD86 (FUN-1)- R-phycoerythrin:Cyanine-5.18 (PE-Cy5) (BD Pharmingen), anti-HLA-DR (L243)-PerCP (Becton Dickinson), anti-CD206 (19.2)-APC (BD Pharmingen), mouse anti-MMP-9 (56-2A4)-purified, mouse anti-PPAR-γ (E-8)-purified (BD Pharmingen), goat anti-mouse IgG (H+L)-PE (Beckman Coulter), anti-CD36 (NL07)-APC (BD Pharmingen) and anti-CCL22 (57203)-PE (R&D Systems Inc., Minneapolis, MN, USA).

Three and four color flow cytometric analyses were carried out using a FACS Calibur flow cytometer (Becton Dickinson) and CellQuest Software (Becton Dickinson).

Statistical Analysis

The paired *t*-test was used to analyze the results. $P < 0.05$ was considered as statistically significant.

Results

The gating and frequencies of CD94+ cells in CD3−CD56+ NK cells are shown in Fig. 1.

Table I shows changes in specific cell percentages before and after HIVIg therapy. Percentages of natural killer T (NKT) cells detected as CD3+CD56+ increased after HIVIg therapy, but without a statistical significance ($P = 0.08$). When we examined percentages of NKT cells as Va24+Vb11+ cells/CD3+ CD4−CD8− cells, the percentages (mean ± S.D., 0.59 ± 0.65%) did not significantly change after HIVIg therapy (0.41 ± 0.51%). Percentage of NK cells detected as CD3−CD56+ cells, CTLs detected as CD3+CD8+ cells, or Tregs detected as CD4+CD25+ cells did not significantly change after HIVIg therapy. Percentages of macrophages detected as CD68+ cells increased after HIVIg therapy, but without a statistical significance ($P = 0.06$).

Cell percentages of CD3−CD56+ NK cells expressing perforin or CD158a did not change after HIVIg therapy. However, percentages of NK cells expressing CD94 significantly ($P = 0.01$) increased after

Table I Changes in Specific Cell Percentages

	Before HIVIg (%)	After HIVIg (%)	*P*-value
Natural killer T cells/lymphocytes	4.5 ± 2.9	6.0 ± 4.3	0.08
Natural killer cells/lymphocytes	8.7 ± 3.6	9.0 ± 3.0	NS
Cytotoxic T cells/lymphocytes	21.7 ± 7.4	21.7 ± 6.7	NS
Regulatory T cells/lymphocytes	18.3 ± 7.4	17.6 ± 7.3	NS
Macrophages/all cells	4.8 ± 2.2	6.9 ± 1.9	0.06

HIVIg, a high dose of intravenous immunoglobulin therapy; (Mean ± S.D.).
NS, not significant.

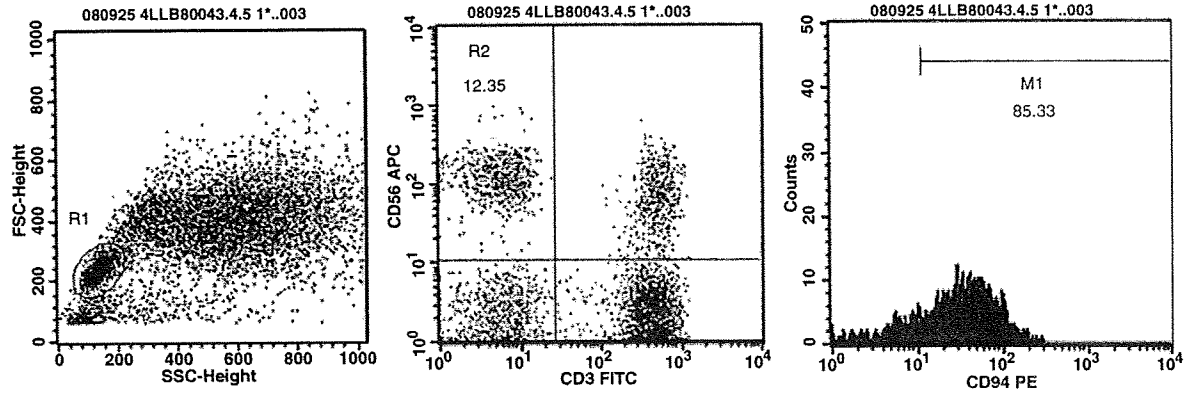

Fig. 1 The gating and frequencies of CD94+ cells in CD3−CD56+ natural killer cells. The peripheral blood was stained with CD3, CD56 and CD94 monoclonal antibodies, as described in Subjects and Methods. A gate (R1) was set on lymphocytes by characteristic forward scatter (FSC) and side scatter (SSC). The analysis gate (R2) was set for CD3−CD56+ lymphocytes and they were further identified by R-phycoerythrin fluorescence conjugating to anti-CD94 monoclonal antibody. CD94+ natural killer cells were detected as a distinct population as marker 1 (M1).

Table II Changes in Percentages of CD3−CD56+ Natural Killer Cells Expressing Specific Molecules

	Before HIVIg (%)	After HIVIg (%)	*P*-value
CD3−CD56+ natural killer cells			
Perforin	89.9 ± 4.7	89.4 ± 4.8	NS
CDI58a	21.7 ± 5.2	22.6 ± 4.6	NS
CD94	58.8 ± 21.4	71.0 ± 17.6	0.01

HIVIg, a high dose of intravenous immunoglobulin therapy (Mean ± S.D.)
NS, not significant.

HIVIg therapy (Table II). When two abortion cases with fetal chromosome abnormality were excluded, percentages of NK cells expressing CD94 more significantly ($n = 6$, $P = 0.003$) increased after the therapy. Fig. 2 shows individual changes in percentages of CD94+ NK cells.

Cell percentages of CD3+CD8+ CTLs expressed perforin in similar ratio before and after HIVIg therapy. Percentages of CD3+CD8+ CTLs expressing CD28 decreased after HIVIg therapy, but with a borderline significance ($P = 0.05$) (Table III).

Cell percentages of CD4+CD25+ Tregs expressing Foxp3, CD28, CD152, or CCR4 did not change after HIVIg (Table IV).

No change was observed in the percentages of CD68+ macrophages expressing CD80, CD86, MMP9, CD206, CD163, HLA-DR, PPAR-γ, CD36, or CCL22 after HIVIg therapy (Table V).

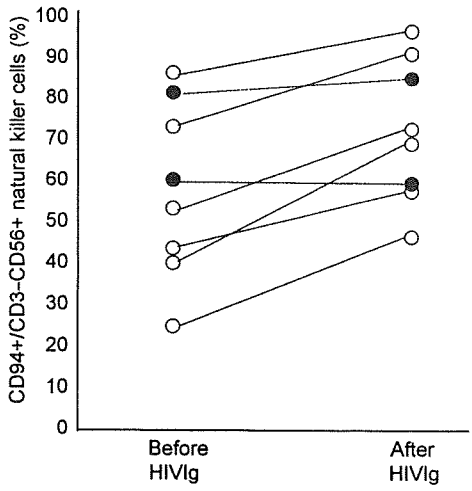

Fig. 2 Changes in percentages of CD94+ natural killer cells; HIVIg, a high dose of intravenous immunoglobulin therapy. Open circles indicate women whose pregnancies ended in live births ($n = 6$); and closed circles indicate women whose pregnancies ended in spontaneous abortion ($n = 2$).

Discussion

The possible pharmacodynamic mechanisms of HIVIg efficacy for clinical use involve interaction of anti-idiotype antibodies with pathologic antibodies, suppression of inflammation, and modification of Fc receptor, T cell, B cell or macrophage functions.[4] We for the first time reported possible efficacy of HIVIg therapy in severe cases of RSA.[13,14] However, immunological mechanisms of HIVIg therapy in RSA have been poorly elucidated.

Concerning NK cell abnormality in spontaneous abortion (SA), it was reported that peripheral NK cell activities were abnormally elevated prior to conception and during early pregnancy in RSA women whose pregnancies were destined to end in SA with normal fetal chromosome karyotype (SANC), but this NK cell accentuation was not detected in RSA women with spontaneous abortion with abnormal fetal chromosome karyotype (SAAC).[17,18] Recently, we found decreased expression of CD94 molecules, which are inhibitory receptors on NK cells, in the decidua of women with sporadic SANC when compared with women with sporadic SAAC or women with induced abortions.[19] These results suggested that the high activity and decrease in inhibitory receptor expression of NK cells might be causally associated with RSA as well as spontaneous abortion.

We previously demonstrated that peripheral NK cell activity was effectively suppressed by HIVIg therapy in RSA women,[14] and demonstrated that expression of inhibitory CD94 was inversely correlated with expression of cytotoxic molecule, perforin on NK cells in the deciduae.[19] In this study, we found no change in peripheral NK cell percentages, but

Table III Changes in Percentages of CD3+ CD5+ Cytotoxic T cells Expressing Specific Molecules

	Before HIVIg (%)	After HIVIg (%)	*P*-value
CD3+CD8+ cytotoxic T cells			
Perforin	24.5 ± 8.4	28.5 ± 11.0	NS
CD28	77.0 ± 5.5	70.9 ± 9.7	0.05

HIVIg, a high dose of intravenous immunoglobulin therapy (mean ± S.D.)
NS, not significant.

Table IV Changes in Percentages of CD4+CD25+ Regulatory T cells Expressing Specific Molecules

	Before HIVIg (%)	After HIVIg (%)	*P*-value
CD4+CD25+ regulatory T cells			
Foxp3	15.6 ± 8.4	15.7 ± 6.2	NS
CD28	99.2 ± 1.3	99.0 ± 1.2	NS
CD152	14.1 ± 12.3	15.5 ± 8.8	NS
CCR4	51.0 ± 7.6	49.9 ± 7.9	NS

HIVIg, a high dose of intravenous immunoglobulin therapy; (mean ± S.D.).
NS, not significant.

Table V Changes in percentages of CD68+ macrophages expressing specific molecules

	Before HIVIg (%)	After HIVIg (%)	*P*-value
CD68+ macrophages			
CD80	2.8 ± 2.1	2.3 ± 1.5	NS
CD86	77.2 ± 15.3	74.5 ± 15.1	NS
MMP9	24.4 ± 40.2	17.5 ± 30.0	NS
CD206	0.9 ± 1.1	1.1 ± 1.7	NS
CD163	1.6 ± 2.2	1.8 ± 1.9	NS
HLA-DR	77.6 ± 9.4	75.6 ± 13.4	NS
PPAR-γ	9.7 ± 9.3	7.3 ± 6.8	NS
CD36	95.4 ± 5.7	95.2 ± 3.3	NS
CCL22	3.8 ± 4.2	3.8 ± 2.3	NS

HIVIg, a high dose of intravenous immunoglobulin therapy: (mean ± SD).
NS, not significant.

found that expression of inhibitory CD94 on NK cells increased after HIVIg therapy. The expression of inhibitory CD94 was more significantly enhanced by the therapy in six women with live births. Conversely, CD94 expression showed no or little increase in two women with SAAC. The increase in CD94 expression on NK cells resulting from the therapy might be related to desirable fetal prognosis and pharmacodynamic mechanisms of HIVIg efficacy. However, in this study, we experienced no SANC. If we could understand changes in CD94 expression among enough number of women with SANC and SAAC, we would discuss whether an increase in CD94 expression predicted fetal prognosis, or was merely a reflection of HIVIg therapy. It is thought that the activity/cytotoxicity of NK cells results from expression balances of various activating receptors and inhibitory receptors on NK cells.[20–22] Inhibitory

receptor complexes (CD94-NKG2A) belong to the C-type lectin superfamily and contain ITIM sequences in their cytoplasmic tails.[22] HIVIg therapy increases the expression of the inhibitory CD94 receptors and subsequently suppresses NK cell activity/cytotoxicity in the body of RSA women. This is a hypothetical mechanism of possible efficacy of HIVIg therapy for RSA.

It is known that the number of NKT cells increases in the deciduae during early pregnancy;[23] and NKT cells may control the Th cell function at the materno-fetal interface through the production of IFN-γ and IL-4.[24] One report demonstrated that increased number of NKT cells in women with RSA or implantation failure was ameliorated by IVIg therapy, leading to successful pregnancy outcome.[25] In this study, however, NKT cell percentages were not significantly changed by HIVIg therapy.

CD3+CD8+ CTLs may play a role in RSA etiologies. We reported increased expression of perforin on CTLs in the deciduae of women with sporadic SANC when compared with women with sporadic SAAC or women with induced abortions. The expression of perforin on CTLs was inversely correlated with expression of inhibitory CD94 on NK cells in the deciduae.[19] In this study, we measured expression of perforin and CD28 on CTLs. CD28 is a ligand of CD80 and CD86 on antigen presenting cells, and can transduce an activating signal. CD28 expression on CTLs decreased after HIVIg therapy, but with a borderline significance ($P = 0.05$). HIVIg therapy may decrease CD28 expression and subsequently suppress activating signal transduction on CTLs in RSA women. This hypothesis should be further clarified. We measured expression of Treg associated molecules including FOXP3, CD28, CD152 (CTLA-4), and CCR4 in CD4+CD25+ T cells. HIVIg therapy did not cause significant changes in expression percentages of these molecules.

Using a mouse model of immunological reproductive failure, we recently demonstrated that intraperitonial injection of a high dose of immunoglobulin restored the fecundity.[15] Additionally, we found that spleen cells adoptively transferred from immunoglobulin injected donors to recipient mice of reproductive failure restored the fecundity. CD11b+ macrophages transferred from donor mice accumulated selectively in the placentae of recipient mice.[15] Therefore, we expected that macrophages might play a key role in mechanisms of HIVIg efficacy in RSA women. In this study, percentages of macrophages increased after HIVIg therapy, but without statistical significance ($P = 0.06$). Expression of macrophage associated molecules including CD80, CD86, MMP9, CD206, CD163, HLA-DR, PPAR-γ, CD36, or CCL22 in the peripheral blood was not changed by HIVIg therapy. Further investigations are needed.

In this study, we experienced desirable pregnancy outcome, i.e., live births after HIVIg therapy in six patients who had a history of four or more abortions of unexplained etiology. We performed the therapy only once during their early pregnancies; and no additional infusion of immunoglobulin was needed. We believe that HIVIg as immune modifier is effective when this therapy is performed during early pregnancy. Severe RSA cases may have immunologic abnormality as its etiology that can be corrected by HIVIg in early pregnancy.

Acknowledgments

This work was supported in part by a Grant-in-Aid H20-kodomo-ippan-002 from the Ministry of Health, Labor and Welfare of Japan.

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Outcome of pregnancy in patients with isolated proteinuria

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Current Opinion in Obstetrics and Gynecology
2009, 21:491–495

Purpose of review

The outcome of pregnancy in patients with isolated proteinuria is believed to be favorable. However, whether women with isolated proteinuria are at risk for progressing to preeclampsia has not been extensively studied.

Recent findings

The amount of proteinuria is thought to increase in the early third trimester, irrespective of whether preeclampsia has been diagnosed. A dipstick urinalysis has a poor sensitivity (ranging from 22 to 86%) for the detection of significant proteinuria (≥0.3 g/day). Measurements of the levels of circulating angiogenic factors such as soluble fms-like tyrosine kinase 1, soluble endoglin, vascular endothelial growth factor, and placental growth factor suggest that gestational proteinuria is a mild variant of preeclampsia. In one study, women with isolated proteinuria (≥0.3 g/day) were found to be more likely to progress to preeclampsia than women with isolated hypertension. A considerable number of women with eclampsia exhibited proteinuria alone during their last antenatal visit performed within a week prior to their first convulsion.

Summary

The outcome of women with a retrospective diagnosis of gestational proteinuria is generally favorable. However, a considerable number of women with isolated proteinuria develop hypertension and progress to preeclampsia. Therefore, the statement that the 'outcome of pregnancy in patients with isolated proteinuria is favorable' is misleading. Physicians should be aware of this type of preeclampsia when counseling patients. One possible explanation for the difficulty in diagnosing this form of preeclampsia might be the low sensitivity of the dipstick urinalysis technique for the detection of significant proteinuria.

Keywords

angiogenic factor, antithrombin activity, eclampsia, gestational proteinuria, preeclampsia

Curr Opin Obstet Gynecol 21:491–495

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Introduction

The classic clinical presentation of preeclampsia is initial hypertension and subsequent proteinuria. However, whether some pregnant women who initially exhibit proteinuria subsequently develop hypertension has not been extensively studied. The clinical outcomes of such women with gestational proteinuria, defined as women who exhibit transient proteinuria of at least 0.3 g/day appearing at or after 20 weeks of gestation and disappearing by 12 weeks postpartum, are believed to be favorable, and proteinuria is not thought to be independently predictive of an adverse outcome [1].

Outcome of pregnancy in women with isolated proteinuria (gestational proteinuria) diagnosed at 12 weeks postpartum

On the basis of the current criteria adopted in many countries, women with proteinuria alone are not diagnosed as having preeclampsia until they also exhibit hypertension [2,3]; those who have not developed hypertension are diagnosed as having had gestational proteinuria [4]. Thus, gestational proteinuria is a retrospective diagnosis. Indeed, the outcomes of women who exhibit transient proteinuria alone (defined as gestational proteinuria in this article) do not differ largely from the outcomes of healthy controls. Holston *et al.* [5••] observed no significant differences in the duration of pregnancy, the rate of preterm delivery, the incidences of gestational diabetes mellitus and renal dysfunction, the mean birth weight, the rates of small-for-gestational-age infants and perinatal deaths, and the need for neonatal intensive care when 108 women with gestational proteinuria were compared with 1564 controls. In four independent studies [5••,6•,7••,8], the reported numbers of gestational weeks at the time of delivery in women with gestational proteinuria were 36.1 ± 3.3 ($n = 7$), 38.2 ± 1.4 ($n = 18$), 39.4 ± 1.0 ($n = 10$), and 39.4 ± 2.0 ($n = 108$), and the outcomes of the pregnancies were consistently better than

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those of women with preeclampsia. Among these studies, the reported numbers of gestational weeks at the onset of proteinuria were 34.2 ± 2.4 and 34.6 ± 3.8 for women who delivered at gestational week 36.1 ± 3.3 [6•] and 38.2 ± 1.4 [7••], respectively.

What is gestational proteinuria?

Whether gestational proteinuria is a variant of preeclampsia (or a preceding stage of preeclampsia) or merely a sign of physiological alterations in kidney function that are not associated with preeclampsia remains uncertain.

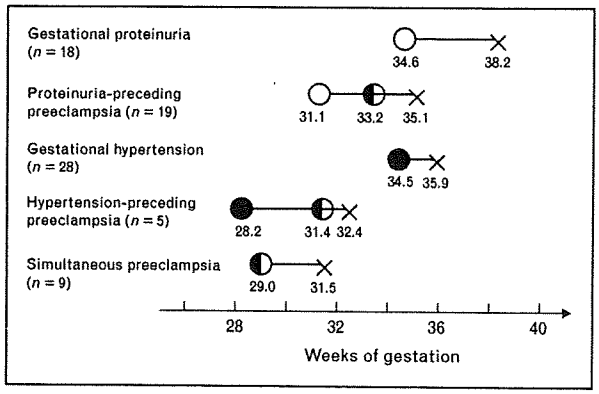
The placental secretion of excessive quantities of antiangiogenic proteins such as soluble fms-like tyrosine kinase 1 (sFlt-1) and soluble endoglin (sEng) into maternal blood, which causes widespread maternal endothelial dysfunction, is thought to be the final common pathway that leads to preeclampsia [9,10,11•]. Women with preeclampsia have increased serum concentrations of sFlt-1 and sEng and reduced concentrations of free vascular endothelial growth factor (VEGF) and free placental growth factor (PlGF), which are proangiogenic proteins that are bound and neutralized by sFlt-1. Because women with gestational hypertension appear to have similar, but modest, alterations in circulating angiogenic proteins [10], Holston *et al.* [5••] hypothesized that gestational proteinuria might be similar to a mild form of preeclampsia and suggested that it might be accompanied by similar alterations in circulating angiogenic factors. Their hypothesis was subsequently verified: compared with gestational age-matched controls, the PlGF level began to decrease beginning 6–8 weeks before the onset of proteinuria. Although the sFlt-1 and sEng concentrations were elevated 1–2 weeks before the onset of proteinuria, these elevations were modest and transient. Similar findings have also been reported by other groups [6•,8]. The concentrations of PlGF and sFlt-1 in women with gestational proteinuria were intermediate between those in controls and in women with preeclampsia in a study by Masuyama *et al.* [8]. The incidences of a high sFlt-1:PlGF ratio (>95th percentile value), a low PlGF level (<5th percentile value), and a high sEng level (>95th percentile value) in women with gestational proteinuria were 57, 29, and 86%, respectively, whereas those in women with preeclampsia were 94, 77, and 88%, respectively, in a study by Ohkuchi *et al.* [6•]. On the basis of the changes in these predictive parameters of preeclampsia, the authors concluded that gestational proteinuria appears to be a mild variant of preeclampsia or might represent subclinical preeclampsia [5••,6•].

An elevation in the serum urate level occurs in hypertensive pregnancies. However, in a study by Morikawa *et al.* [7••], the reported serum urate level of 5.6 ± 1.5 mg/dl among women with gestational proteinuria tended to be higher than that of 4.8 ± 1.6 mg/dl among women with gestational hypertension but was significantly lower than that of 7.2 ± 1.6 mg/dl among women with preeclampsia [7••]. The incidence of a high serum urate level (>7.0 mg/dl) was 22% for women with gestational proteinuria, 0.0% for women with gestational hypertension, and 67% for women with preeclampsia [7••]. Antithrombin activity is another parameter that is known to be decreased in hypertensive pregnancies [12]. The incidence of a low antithrombin activity (<70%) was 22% for women with gestational proteinuria, 11% for women with gestational hypertension, and 67% for women with preeclampsia [7••]. Although the incidence of a low antithrombin activity of less than 70% among women with uncomplicated pregnancies was not described in the study by Morikawa *et al.* [7••], the rate was less than 3.0% (personal communication). These results may support the notion that gestational proteinuria is a mild variant of preeclampsia or might represent subclinical preeclampsia.

Do women with isolated proteinuria often progress to preeclampsia?

Apparently, some women exhibit transient proteinuria during pregnancy; changes in biological parameters such as the sFlt-1, PlGF, sEng, and uric acid levels and the antithrombin activity, as mentioned above, suggest that these women might have a high risk of developing preeclampsia. Thus, determining whether women with isolated proteinuria actually develop preeclampsia more frequently than women without proteinuria is a concern. If so, the statement that 'the outcome of women with isolated proteinuria is favorable' would be incorrect and misleading from a prospective viewpoint. Although a case report of a woman who initially showed proteinuria and developed hypertension 2 days later has been made [13•], to the best of our knowledge, no case series have dealt with this issue other than a study by Morikawa *et al.* [7••]. They reviewed the medical records of 79 women who developed proteinuria of more than 3.0 g/day and/or hypertension at or after 20 weeks of gestation, focusing on the gestational week at which proteinuria (>0.3 g/day) and/or hypertension developed. Thirty-seven (47%) women exhibited new-onset proteinuria in the absence of hypertension at 32.8 ± 4.8 weeks of gestation, 33 (42%) exhibited new-onset hypertension in the absence of proteinuria at 33.5 ± 4.7 weeks of gestation, and nine (11%) exhibited both proteinuria and hypertension simultaneously at 29.0 ± 4.3 weeks of gestation [7••]. As many as 19 (51%) of the 37 women with isolated proteinuria later developed hypertension at 33.2 ± 4.7 weeks of gestation (Fig. 1), whereas only five (15%) of the 33 women with a presumptive diagnosis of gestational hypertension progressed to preeclampsia ($P = 0.022$). No significant differences in the number of gestational weeks

Figure 1 Mean gestational week at which proteinuria (○), hypertension (●), and delivery (×) developed/occurred in five groups of women



Among 37 women with isolated proteinuria, 19 women who developed hypertension later (proteinuria-preceding preeclampsia) showed proteinuria significantly earlier than the remaining 18 women who remained normotensive (gestational proteinuria). Data from Morikawa *et al.* [7••].

at the time of delivery (36.6 ± 3.4 vs. 35.4 ± 3.5 weeks), the rate of preterm birth at less than 37 weeks (35.1 vs. 42.4%), the rate of fetal growth restriction (21.6 vs. 24.2%), abruptio placenta (10.8 vs. 9.1%), or hemolytic anemia, elevated liver enzymes, and low platelet count (HELLP) syndrome (2.7 vs. 3.3%) were observed between the two starting cohorts of 37 women with isolated proteinuria and the 33 women with a presumptive diagnosis of gestational hypertension. However, in comparison with 19 women diagnosed as having proteinuria preceding preeclampsia and 18 women diagnosed as having gestational proteinuria at 12 weeks postpartum, significant differences were observed in the rate of preterm birth at less than 37 weeks (57.9 vs. 11.1%), birth weight (2142 ± 775 vs. 2797 ± 524 g), antithrombin activity (77.4 ± 13.0 vs. 89.7 ± 18.4%), and the rate of antithrombin activity of less than 70% (42.1 vs. 22.2%). Thus, some women with isolated proteinuria subsequently develop hypertension, and the outcomes of these women who later develop hypertension are worse than that of women with only proteinuria [7••]. Although these results are not obtained from a prospective cohort study, these findings suggest that women with isolated proteinuria may be more likely to progress to preeclampsia than women with a presumptive diagnosis of gestational hypertension, and that approximately half of the women with isolated proteinuria may progress to preeclampsia. The reported rate of progression to preeclampsia, which was 15% (5/33) among women with a presumptive diagnosis of gestational hypertension [7••], was consistent with the result of an earlier study by Saudan *et al.* [14], in which 15–25% of women who were initially diagnosed as having gestational hypertension eventually developed preeclampsia.

Table 1 Appearance of hypertension and proteinuria prior to the first convulsion in women with eclampsia

	1992	2005–2006
Number of patients with eclampsia	325	214
Proteinuria alone	32 (9.8%)	16 (7.5%)
Hypertension alone	71 (21.8%)	20 (9.3%)
Both	186 (57.2%)	81 (37.9%)

The number of women who had hypertension (DBP ≥90 mmHg), proteinuria (≥+1 on dipstick or ≥0.3 g/day), or both during the last antenatal visit within 1 week prior to their first seizure is indicated. Data from Douglas and Redman [15] and Knight *et al.* [16].

Clinicians must be able to counsel pregnant women who develop proteinuria appropriately. The outcome of women with a retrospective diagnosis of 'gestational proteinuria' is indeed favorable, but counseling must be done in a prospective fashion. The statement 'proteinuria is not independently predictive of adverse outcome' [1] is misleading. This conclusion is further supported by the following two studies [15,16]: in the UK, 32 (10%) of 325 women with eclampsia in 1992 and 16 (7.5%) of 214 women with eclampsia between February 2005 and February 2006 exhibited proteinuria alone at the time of their last antenatal visit within 1 week of their first convulsion (Table 1).

Preeclampsia/eclampsia may occur in postpartum women with undiagnosed preeclampsia after hospital discharge [17•]. Yancey *et al.* [17•] reviewed 22 patients who initially presented at an emergency department (ED) during the postpartum period after hospital discharge with complaints such as headache and visual changes: 12 (55%) women had not been diagnosed with preeclampsia in the ante or peripartum period; the time of presentation ranged from 3 to 10 days postpartum, with a median time of 5 days; the initial DBP recorded in the ED ranged from 60 to 114 mmHg, and the DBP was less than 90 mmHg in five (23%) women; 21 (95%) women had either proteinuria, an elevated uric acid level, or an abnormal liver function test.

Thus, hypertension and proteinuria clearly do not necessarily appear together either at the onset of the syndrome or prior to complications, and a considerable number of women with eclampsia have proteinuria alone prior to their first seizure. In addition, it is apparent that women with isolated proteinuria are more likely to develop eclampsia than women with neither proteinuria nor hypertension. Thus, the general belief that 'proteinuria develops late during the course of preeclampsia' might not be correct.

Natural history of new proteinuria in pregnancy and problems with the detection of significant proteinuria

The amount of proteinuria appears to increase during pregnancy. Poon *et al.* [18•] found that the first trimester

median urine albumin concentration and the median albumin-to-creatinine ratio were already significantly elevated in women who developed preeclampsia compared with unaffected individuals. Bar *et al.* [19] examined urinary microalbuminuria levels longitudinally and demonstrated a statistically significant increase in the albumin excretion rate in the second and third trimesters compared with the first trimester. Franceschini *et al.* [20] found that the albumin : creatinine ratio in the urine collected at around 27 weeks of gestation was strongly associated with preterm birth in a dose–response fashion. This association was present for both spontaneous and medically induced preterm birth, and a risk was observable at levels of albuminuria commonly considered to be within the normal range of nonpregnant women and at levels much lower than those detectable using the urine dipstick method, which is commonly used to detect preeclampsia. Gordon *et al.* [21] reported that proteinuria increases from a mean of 1.71 ± 1.33 g/day during the first trimester to a mean of 4.82 ± 4.7 g/day during the third trimester in women with diabetic nephropathy, irrespective of whether the patient is diagnosed as having preeclampsia. Schiff *et al.* [22] reported that proteinuria increases in most women with severe preeclampsia who are managed conservatively (median increase of 660 mg over each 24-h period). Morikawa *et al.* [7••] reported that proteinuria increases with advancing gestation in all three groups of women with gestational proteinuria, proteinuria preceding preeclampsia, or hypertension preceding preeclampsia. These results suggest that protein excretion in the urine begins at a level much lower than 0.3 g/day and continues to increase to at least 0.3 g/day in some women. If this is the case, an appropriate cut-off level for the albumin : creatinine ratio or the protein : creatinine ratio determined at a certain stage of pregnancy might efficiently predict the development of preeclampsia, as has been previously reported [18•,19,20].

Screening for significant proteinuria ($\geq$0.3 g/day) has traditionally been performed using the urine dipstick method, but this method is prone to considerable error. Prior studies [23–27] have reported that a dipstick urinalysis has varying degrees of accuracy, with sensitivities ranging from 22 to 86%. Thus, a considerable number of women with significant proteinuria may have been overlooked and judged not to have proteinuria, leading to the general belief that 'proteinuria develops late in the course of preeclampsia'.

At present, a 24-h urine collection is considered to be the gold standard for quantifying proteinuria. However, a 24-h urine collection is time-consuming and frequently inaccurate, and therefore not a precise measure of proteinuria [28••]. The completeness of a 24-h urine collection should not be assessed by urine volume but rather by urinary creatinine excretion. Twenty-four-hour urinary creatinine excretion reflects muscle mass, and excretion is relatively constant over time in a given person [29]. Although the use of the protein : creatinine ratio to estimate 24-h protein excretion for the diagnosis of preeclampsia has been controversial, two studies [30,31••] describing meta-analyses showed pooled sensitivities of 90 and 84% and specificities of 78 and 76% using various protein : creatinine ratio cut-offs for the detection of significant proteinuria.

Conclusion

Some women with preeclampsia initially exhibit proteinuria followed by the subsequent development of hypertension. Women with isolated proteinuria should be counseled as to the possibility of proteinuria preceding preeclampsia. One possible explanation for the difficulty in diagnosing this type of preeclampsia might be the low sensitivity of the dipstick urinalysis technique. Larger prospective cohort studies using the protein : creatinine ratio and focusing on the gestational week at which proteinuria and/or hypertension develop are needed to determine how often this form of preeclampsia occurs and to search for an efficient protein : creatinine ratio cut-off value for the prediction of adverse outcome.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 543).

1 Airoldi J, Weinstein L. Clinical significance of proteinuria in pregnancy. Obstet Gynecol Surv 2007; 62:117–124.

2 Report of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy. Am J Obstet Gynecol 2000; 183:S1–S22.

3 Brown MA, Hague WM, Higgins J, *et al.* Australian Society of the Study of Hypertension in Pregnancy. The detection, investigation and management of hypertension in pregnancy: full consensus statement. Aust N Z J Obstet Gynaecol 2000; 40:139–155.

4 Sato K. New name, definition and classification of 'pregnancy-induced hypertension' which aimed at international universality. Nippon Syusanki Shinseiji Gakkai Zasshi 2005; 41:667–674.

5 •• Holston AM, Qian C, Yu KF, *et al.* Circulating angiogenic factors in gestational proteinuria without hypertension. Am J Obstet Gynecol 2009; 200:392.e1–392.e10.
This study provides data on circulating PlGF, sFlt-1, and sEng in women with gestational proteinuria; the changes in these parameters were similar to those in women with preeclampsia, but more modest.

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This study demonstrated that the incidence rates of a high sFlt-1:PlGF ratio (95th percentile value), a low PlGF level (5th percentile value), and a high sEng level (95th percentile value) in women with gestational proteinuria were 57, 29, and 86%, respectively, whereas those in women with preeclampsia were 94, 77, and 88%, respectively.

7 •• Morikawa M, Yamada T, Yamada T, *et al.* Pregnancy outcome of women who developed proteinuria in the absence of hypertension after mid-gestation. J Perinat Med 2008; 36:419–424.
This observational study is very interesting; it demonstrated that some women who initially develop proteinuria subsequently develop hypertension and suggested that women with isolated proteinuria are more likely to develop preeclampsia than women with a presumptive diagnosis of gestational hypertension.

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10 Levine RJ, Lam C, Qian C, *et al.* Soluble endoglin and other circulating antiangiogenic factors in preeclampsia. N Engl J Med 2006; 355:992–1005.

11 • Mutter WP, Karumanchi A. Molecular mechanisms of preeclampsia. Microvasc Res 2008; 75:1–8.
This study is a systematic review of the role of angiogenic and antiangiogenic proteins in the molecular mechanisms of preeclampsia.

12 Weenink GH, Treffers PE, Vijin P, *et al.* Antithrombin III levels in preeclampsia correlate with maternal and fetal morbidity. Am J Obstet Gynecol 1984; 148:1092–1097.

13 • Andrus SS, Wolfson AB. Postpartum preeclampsia occurring after resolution of antepartum preeclampsia. J Emerg Med 2008. [Epub ahead of print]
This study documents a woman who initially exhibited proteinuria and subsequently developed hypertension.

14 Saudan P, Brown MA, Buddle ML, Jones M. Does gestational hypertension become preeclampsia? Br J Obstet Gynaecl 1998; 105:1177–1184.

15 Douglas KA, Redman CWG. Eclampsia in the United Kingdom. BMJ 1994; 309:1395–1400.

16 Knight M, UKOSS. Eclampsia in the United Kingdom 2005. Br J Obstet Gynaecol 2007; 114:1072–1078.

17 • Yancey LM, Withers E, Bakes K, Abbott J. Postpartum preeclampsia: emergency department presentation and management. J Emerg Med 2008. [Epub ahead of print]
This study describes 22 women with preeclampsia/eclampsia who initially presented at an ED during the postpartum period after discharge; 12 (55%) women had not been diagnosed as having preeclampsia during the ante or peripartum period, and the DBP at presentation was less than 90 mmHg in five (23%) women.

18 • Poon LC, Kametas N, Bonino S, *et al.* Urine albumin concentration and albumin-to-creatinine ratio at 11+0 to 13+6 weeks in the prediction of preeclampsia. BJOG 2008; 115:866–873.
This study showed that the first trimester median urine albumin concentration and the median albumin-to-creatinine ratio were already significantly elevated among women with preeclampsia compared with those in the unaffected group.

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20 Franceschini N, Savitz DA, Kaufman JS, Thorp JM. Maternal urine albumin excretion and pregnancy outcome. Am J Kidney Dis 2005; 45:1010–1018.

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25 Waugh JJ, Clark TJ, Divakaran TG, *et al.* Accuracy of urinalysis dipstick techniques in predicting significant proteinuria in pregnancy. Obstet Gynecol 2004; 103:769–777.

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28 •• Côté AM, Firoz T, Mattman A, *et al.* The 24-h urine collection: gold standard or historical practice? Am J Obstet Gynecol 2008; 199:625.e1–625.e6.
The objective of this study was to determine the completeness of 24-h urine collection; the study demonstrated that this method is frequently inaccurate and not a precise measure of proteinuria.

29 Perrone RD, Madias NE, Levey AS. Serum creatinine as an index of renal function: new insights into old concepts. Clin Chem 1992; 38:1933–1953.

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31 •• Côté AM, Brown MA, Lam E, *et al.* Diagnostic accuracy of urinary spot protein:creatinine ratio for proteinuria in hypertensive pregnant women: systematic review. BMJ 2008; 336:1003–1006.
This study is a systematic review of reports on the diagnostic accuracy of the urinary spot protein : creatinine ratio for the diagnosis of significant proteinuria; the study concluded that the spot protein : creatinine ratio is a reasonable 'rule-out' test for detecting proteinuria levels of 0.3 g/day or more during pregnancy.