with the τ of zero asymptote in 20 children. Further validation was required because the τ of zero asymptote may reflect the effect of factors other than changes in LV relaxation [23]. Although our results were in agreement with previous findings subjectively [22], our correlations were not as strong as those previously reported. In addition, our study demonstrated for the first time that e' correlated with logistic tau (τ_L) [24], a more robust form of the time constant of relaxation [25], and with nonzero asymptote (τ_{ME}; Fig. 2). Furthermore, our study established the cutoff values of e' to detect abnormally slow LV relaxation and validated the hypothesis that dobutamine-associated changes in e' reflected the changes in τ in children (Fig. 3). A cutoff value of septal $e' < 6.2$ determined the 90th percentile of τ_L with sensitivity and specificity of 0.83, indicating the usefulness of e' in detecting abnormally slow LV relaxation in children. Of note, the observation of a dobutamine-induced change in e' (Fig. 3) indicates that e' may also be useful for serial monitoring of the change in relaxation in a given patient with heart disease.

Stiffness

In theory, DT should be inversely correlated with the square root of LV stiffness [26]. As shown in Fig. 4, we confirmed a significant inverse relationship between DT and K in our study subjects with heart disease, although the plots were rather scattered. This weak correlation may be explained by several factors. First, this study used approximated volume changes to generate K, resulting in an erroneous calculation of K. Second, mitral E and A waves tend to merge in children because of a more rapid heart rate, which shortens the E deceleration line and may contribute to an erroneous measurement of DT. Finally, DT did not change linearly with a worsening diastolic function. DT was longer in a delayed relaxation pattern than in a normal diastolic pattern, but became shorter as diastolic function progressively worsened [1]. Therefore, the usefulness of DT alone may be quite limited in healthy subjects or in a population without restrictive physiology. By contrast, DT may provide useful information about the characteristics of patients with advanced heart disease. Indeed, DT strongly correlates with pulmonary capillary wedge pressure (PCWP) in adult postinfarction patients [27] with reduced ejection fraction (<35 %), and a short DT indicates poor prognosis in adult patients after a myocardial infarction and heart transplantation [28]. Although reduced DT is associated with poor prognosis in pediatric patients with Duchenne muscular dystrophy [29], the usefulness of DT may be limited in children with preserved diastolic function. The relationship between DT and filling pressure, and the prognostic significance of DT in children require further clarification, and future studies involving a larger number of pediatric patients with advanced heart disease are warranted.

End-diastolic pressure

E/e' correlated better with LV EDP than DT (Fig. 5). Our observations on the significant E/e'-filling pressure relationship (Fig. 5b) in children with various heart diseases (mean age, 3.0 years; Table 1) are somewhat different from the finding of Goldberg et al. [30] that E/e' did not correlate with PCWP in 49 children after heart transplantation (mean age, 11.7 years), or the finding of Border et al. [22] that E/e' did not correlate significantly with LV EDP in 20 children with heart disease (mean age, 6.6 years). However, our results are in agreement with those of Oyamada et al. [31] that E/e' correlated significantly with LV EDP in 48 smaller children (mean age, 9 months) with ventricular septal defects. These discrepancies may be explained by the differences in the numbers of patients, age, heart rate, cardiac geometry [32], loading condition, and range of filling pressure; presence or absence of cardiac denervation; or differences in the measurement of filling pressure (LV EDP vs PCWP). Of note, the present study involved a larger number of patients with various heart diseases, which may be advantageous for assessing the robustness of the relationship. In addition, we demonstrated that the combination of E/e' and DT provided a better estimate of LV EDP. Further study is therefore warranted to develop a better method for predicting LV EDP by using multiple echocardiographic indexes.

Although the overall E/e'-LV EDP relationship was significant, the correlations were not strong and the data were rather scattered. However, E/e' may be more useful for detecting an elevated LV EDP. ROC analysis to detect elevated LV EDP (>12.96 mmHg, 90th percentile of the study population) using E/e', DT, and the combination of the three indexes revealed that E/e' (>16.4) best predicted elevated LV EDP, with an AUC of 0.81 (Fig. 5c). Although the sensitivity of E/e' of 0.71 was less satisfactory for detecting a high LV EDP in children, the specificity of 0.93 was sufficiently high. Therefore, an elevation in LV EDP cannot be excluded even when E/e' is not elevated, whereas an elevation in LV EDP should be strongly considered in children with high E/e' values.

The reported clinical usefulness of E/e' in children included its association with quality of life after repair of tetralogy of Fallot independent of LV systolic function [33], its ability to predict adverse clinical outcomes in children with hypertrophic cardiomyopathy [8], and its association with high-grade cellular rejection in patients who underwent cardiac transplantation [30]. More clinical situations in which E/e' is related to

patient prognosis or disease severity need to be identified in future studies.

Study limitations

Localized problems in the ventricular wall may inhibit proper assessment using TDI. This may be the case in our patients, because most of them underwent surgery (Table 1). Although none of the patients showed apparent abnormality in localized wall motion, the effects of this factor on mitral inflow and annular velocities need to be assessed further in future studies. Another weakness of the tissue-Doppler method is angle dependency. Minimal angulation (<20°) should be present between the ultrasound beam and the plane of cardiac motion [34]. We minimized this disadvantage of angle dependency by using septal e', which has less angle dependency than that of lateral e'. Septal e' is more reliable than lateral e' when minimal angulation of lateral annulus cannot be obtained. In fact, such proper minimal angulation for lateral e', which was performed only under supine position for simultaneous catheterization and thus allowed a limited echocardiographic window, was not obtained in a considerable proportion of the patients in this study. However, there are concerns that septal e' may be affected by right ventricular volume overload or dysfunction, which can be sometimes observed after repair of congenital heart disease such as tetralogy of Fallot [35]. Future large-scale studies should investigate how such right ventricular factors [36] affect the differences between septal and lateral TDI results, as well as the evaluation of diastolic function. Although speckle tracking can overcome these inherent limitations of TDI, speckle tracking is a relatively time-consuming process and is not in routine clinical use in many institutions. We therefore believe that the validations in this study are of major clinical importance, first, because pulse Doppler and tissue-Doppler data can be readily obtained and, second, because they are routine clinical measurements in current echocardiographic evaluation. This study included 11 patients whose Q_p/Q_s was not equal to 1. Q_p/Q_s was previously reported to be negatively correlated with e' but positively correlated with E/e' [31]. Thus, Q_p/Q_s might have affected the results of this study. In subgroup analysis of the patients with $Q_p/Q_s = 1$, weak but significant relationships ($P < 0.05$) remained between e' and relaxation, DT and K, and E/e' and EDP, the main results of this study (data not shown). However, because of the limited number of patients in the subgroup analysis, accumulation of more patients with $Q_p/Q_s = 1$ is needed for further subgroup analysis. This study involved a relatively small number of patients, and did not include patients with severely impaired diastolic function such as dilated cardiomyopathy; therefore, the cutoff values provided in this study need to be refined and fully validated in future multicenter studies involving a larger number of children with normal to severely impaired diastolic function.

A noninvasive echocardiographic assessment using early diastolic mitral inflow and annular velocities provides a clinically relevant assessment of diastolic function in pediatric patients. Although these indexes reflect diastolic function but do not provide an absolute measure of diastolic function, our study provides the basis for the creation of a definitive algorithm for a noninvasive diastolic evaluation that would enable better management of children with heart disease.

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Conflict of interest None.

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ORIGINAL ARTICLE
Cardiovascular Surgery

Usefulness of Cystatin C in the Postoperative Management of Pediatric Patients With Congenital Heart Disease

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Background: The characteristics of the renal marker cystatin C (Cys-C) in association with the postoperative management of children with congenital heart disease (CHD) remain unclear.

Methods and Results: Serum Cys-C and creatinine (Cr) levels were measured preoperatively and on the third postoperative day in 53 consecutive CHD patients (age, 1 day–11 years). On the third postoperative day, the patients were divided into 2 groups: the clinically severe group, requiring continuous infusion of diuretic drugs or peritoneal dialysis; and the non-severe group, composed of those without such needs. Preoperative Cys-C level decreased with age (by month) during the first year of life and remained almost constant thereafter, while Cr level increased with age. The Cys-C ratio (Cys-C level on the third postoperative day/preoperative level) was positively correlated with Cr ratio ($R=0.57$, $P<0.001$). Both Cys-C and Cr levels increased in correlation with the clinical severity of renal impairment. Receiver operating characteristic curve analysis failed to demonstrate an advantage of Cys-C over Cr in detecting severity.

Conclusions: Cys-C may be a useful marker of renal function in terms of hemodynamic status in the postoperative management of CHD, although its superiority over Cr could not be confirmed. Future studies should clarify the role of Cys-C in clinical decision-making and evaluate the relationship of Cys-C with factors that may affect its levels. (*Circ J* 2013; **77:** 667–672)

Key Words: Creatinine; Intensive care; Postoperative state; Renal function

In the postoperative management of pediatric patients with heart disease,[1] cardiac loading conditions[2] and functions change dramatically, sometimes requiring large amounts of diuretics or dialysis. Moreover, drugs that require adjustment according to renal function, such as antibiotics, are often used. Thus, simple and accurate methods of determining renal function are extremely useful in this setting. Renal function is often assessed by measuring the glomerular filtration rate (GFR).[3] Inulin clearance is an accurate method of measuring GFR and is regarded as the international standard. Because this method is cumbersome, however, and requires fluid load, inulin clearance is inappropriate for routine medical practice, especially when repetitive use is required as in the postoperative management of pediatric patients with heart disease. Other assessment methods using exogenous substances are also impractical.[4] Similarly, special diagnostic imaging, such as renal scintigraphy, is also inappropriate.

Creatinine (Cr) clearance based on 24-h urine collection and endogenous Cr level have been used as a surrogate of GFR. Cr clearance, however, is higher than the actual GFR because of tubular excretion of Cr. As GFR decreases, the differences between Cr clearance and GFR become larger.[5] Moreover, because urine collection through voluntary voiding can be difficult for children, placement of an invasive bladder catheter is necessary even in pediatric patients being weaned from intensive care. Although a method of estimating GFR based on Cr level, sex, and body size is frequently used in adults, there is, as yet, no clinically established method for children.[4,6]

Serum Cr level itself can be measured with a single blood test and is now a routine test at many institutions. Because it can be measured rapidly with a small sample at low cost, Cr is a useful and very frequently used marker of renal function. Cr, however, is systemically produced from creatine, and the creatine pool in the body is affected by muscle mass and exercise. In addition, unless GFR decreases to a certain extent, Cr level will not change.[7] These are considered limitations for the application of Cr level as an accurate marker of renal function.

Serum cystatin C (Cys-C), a cysteine protease inhibitor that

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Table. Disease Type vs. Age

	Age <3 months	n	3 months – <1 year	n	Age ≥1 year	n
M/F	9/8	17	6/6	12	15/9	24
BW (kg)	1.8–4.4 (3.0±0.7)		4.6–7.3 (5.8±1.1)		6.5–28.2 (13.5±6.5)	
	HLHS	1	HLHS	3	HLHS	1
	SRV, PA, TAPVC	2	SRV, PS	1	SRV, PS/PA, TAPAC	5
	SRV, AS, CoA	2	cAVSD	1	DORV	3
	SRV, hypoLV, AR	1	TA	1	Ebstein, PA	1
	DORV, TAPVC	1	PA IVS	1	PA IVS	1
	Ebstein, SAS	1	VSD	4	cTGA, PA, MA	1
	Ebstein, PA	1	TGA, VSD, PS	1	TOF, PA, MAPCA	1
	PA, VSD, MAPCA	1			TOF	2
	CoA, VSD	2			VSD	3
	VSD	3			cTGA	1
	TAPVC	1			CoA	1
	PDA	1			ASD	1
					AS, PS	1
					AS	1
					AR	1

Data given as range (mean±SD).
AR, aortic regurgitation; AS, aortic stenosis; ASD, atrial septal defect; BW, body weight; cAVSD, complete atrioventricular septal defect; CoA, coarctation of aorta; cTGA, corrected transposition of great arteries; DORV, double outlet right ventricle; HLHS, hypoplastic left heart syndrome; hypoLV, hypoplastic left ventricle; IVS, intact ventricular septum; MA, mitral atresia; MAPCA, major aortopulmonary collateral arteries; PA, pulmonary atresia; PDA, patent ductus arteriosus; PS, pulmonary stenosis; SAS, subaortic stenosis; SRV, single right ventricle; TA, tricuspid atresia; TAPVC, total anomalous pulmonary venous connection; TOF, tetralogy of Fallot; VSD, ventricular septal defect.

is produced and secreted by nucleated cells in the body, is a GFR marker that does not depend on muscle mass. Cys-C is a low-molecular-weight protein that passes freely through renal glomeruli, and at least 99% of it is reabsorbed and catabolized by proximal renal tubules. Thus, Cys-C may serve as a better indicator of GFR than Cr. Serum Cys-C level can also be determined with a single blood sample. The usefulness of Cys-C as a marker of renal function, mainly in adults, has been reported regularly,[8–10] and Cys-C has also gradually come into use in children.[3,7,11–16] Although Cys-C would be expected to be useful in the postoperative management of pediatric patients with heart disease, detailed studies are limited.

Here, we examined whether Cys-C is potentially a more useful marker of renal function than conventional serum Cr in the postoperative management of pediatric patients with heart disease.

Methods

Patients

This study involved 53 consecutive pediatric patients (29 boys and 24 girls) with congenital heart disease (CHD) who underwent surgery at Saitama Medical University. Their age ranged from 1 day to 11 years (mean±SD, 1.6±2.3 years; median, 0.7 years). **Table** lists heart disease type.

Age Dependency of Cys-C and Cr

Because previous studies suggested that Cys-C and Cr may vary with age in children, we first examined the age dependence of these markers by using preoperative samples. As shown in **Figure 1**, preoperative Cys-C level decreased with age (by month) during the first year of life and remained almost constant thereafter, regardless of age. Preoperative Cys-C ranged from 0.71 mg/L (2 and 5 years old) at the lowest to 2.45 mg/L (25 days old) at the highest. The 5 patients with Cys-C ≥2.0 mg/L were all younger than 3 months. In patients aged ≥1 year, the range was narrow, from 0.71 to 1.30 mg/L (mean, 0.97 mg/L).

Meanwhile, as was the case with Cys-C, preoperative Cr level was high before the age of 3 months and then decreased with age (in months). Unlike Cys-C, Cr level tended to show an age-dependent increase after the first year of life (**Figure 1**). Preoperative Cr level ranged from 0.21 mg/dl (1 year old) to 1.1 mg/dl (12 days old). The Cr level of all patients before surgery was below the 90th centile of the reference range.[16] The increase after the first year of life was age dependent.

Based on these results showing the age-dependent distribution of preoperative serum Cys-C and Cr, all analyses were performed separately in the 3 different age groups (<3 months of age, n=17; age 3 months–<1 year, n=12; and ≥1 year, n=24).

Clinical Severity and Cys-C or Cr

Serum Cys-C and Cr levels were measured again on the third postoperative day, when the large perioperative shifts in hemodynamic and fluid status were resolved in most patients. Based on the clinical findings on the third postoperative day, the patients were divided into 2 groups for analysis of renal function and circulatory status: the clinically severe group, composed of patients who required peritoneal dialysis or continuous i.v. furosemide; and the non-severe group, which included patients without such needs. The requirement for furosemide or peritoneal dialysis was determined by intensive care physicians who were unaware of the study design and were blinded to serum Cys-C level. Peritoneal dialysis was initiated within 2 h after surgery based on a detailed assessment of the complexity of the operation and the hemodynamic status after cardiopulmonary bypass, and independent of the serum Cr level, as previously reported by Dittrich et al.[17] Continuous infusion of furosemide was performed when sufficient diuresis was not obtained with a single dose (0.3–1 mg/kg) or when the poten-

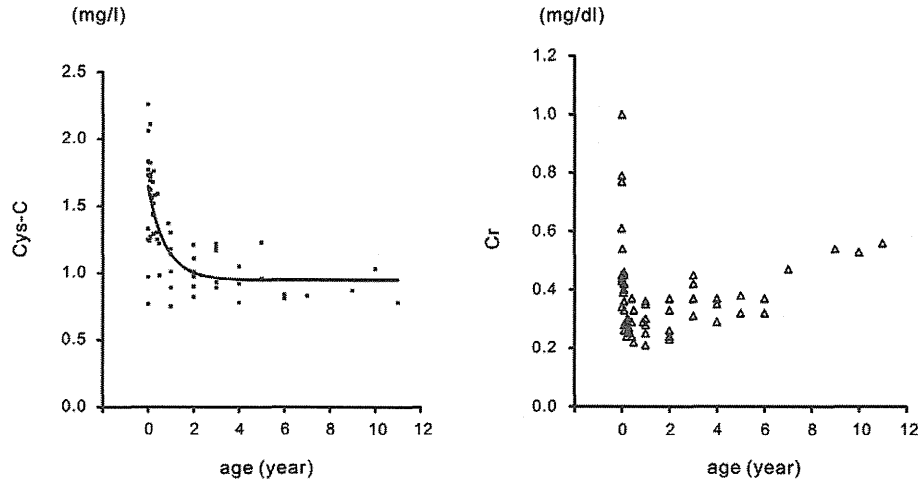

Figure 1. Age-dependent changes in preoperative cystatin C (Cys-C) and creatinine (Cr) level in pediatric patients with heart disease. Preoperative Cys-C level decreased with age (in months) during the first year of life and remained almost constant thereafter. Preoperative Cr level was high before the age of 3 months and then decreased with age (in months). Unlike Cys-C, there was a tendency for an age-dependent increase in Cr after the first year of life.

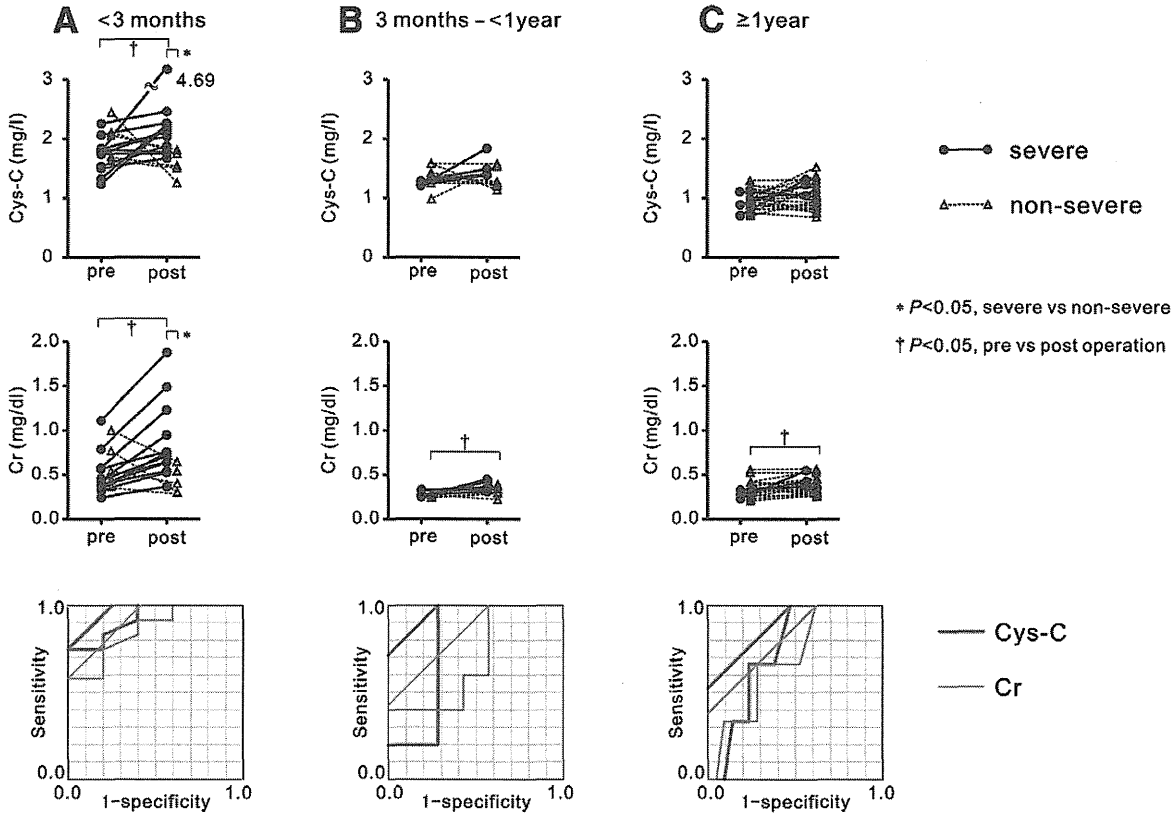

Figure 2. Changes in (**Top row**) cystatin C (Cys-C) and (**Middle row**) creatinine (Cr) before and after surgery and (**Bottom row**) receiver operating characteristic (ROC) curve analyses for detection, by age, of clinically severe postoperative pediatric patients with heart disease. Comparison between the groups classified on the third postoperative day as (●) clinically severe and (△) non-severe indicated statistically significant differences in both Cys-C and Cr in the age group <3 months of age. The (**Bottom row**) ROC curve analyses were performed using the levels on the third postoperative day for detection of clinically severe status. The areas under the ROC curve were larger for Cys-C in all age groups.

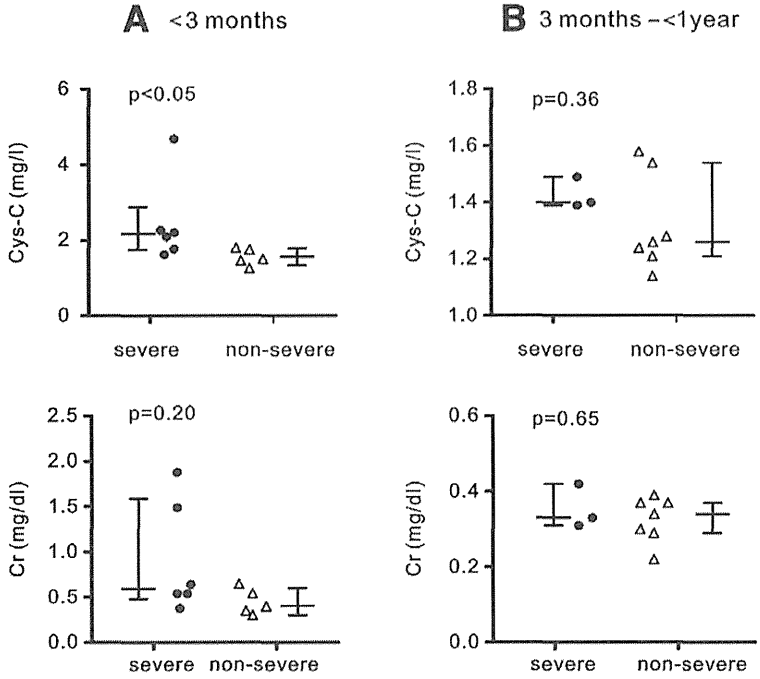

Figure 3. Comparison of cystatin C (Cys-C) and creatinine (Cr) levels on the third postoperative day between the clinically severe group without peritoneal dialysis and the non-severe group. Error bars show median with interquartile range. Cr level did not differ significantly between the severe and non-severe groups, whereas Cys-C level was statistically significantly different between the groups in patients <3 months of age.

tial influence of body fluid variation on hemodynamics caused by a single dose raised concern. We assessed whether the serum level of Cys-C or Cr better reflected the clinical severity.

Postoperative Percent Change in Cys-C and Cr

The ratio of serum Cys-C and Cr levels on the third postoperative day to that before surgery was compared by age group.

Measurement and Analysis

Serum Cys-C was measured using latex-enhanced turbidimetric immunoassay (Iatro Cys-C; Mitsubishi Chemical Medience), and serum Cr was measured with an enzymatic method using TBA-cl 6000, a creatininase-HMMPS method analyzer. Two paired groups were compared using the two-tailed paired t-test, and 2 unpaired groups were compared using the Wilcoxon rank-sum test. Receiver operating characteristic (ROC) curve analysis was performed on the group classified as clinically severe on the third postoperative day. The serum measurements of renal function were performed as part of general pre- and postoperative management and the use of patient data followed institutional regulations. This study was approved by the Institutional Review Board of the International Medical Center, Saitama Medical University.

Results

Clinical Severity and Cys-C or Cr

Clinical severity classification performed on the third postoperative day showed that the patients in the younger group tended to be severe. Of the 17 patients <3 months of age, all but 5 were severe. In the 24 patients aged ≥1 year, only 3 (12.5%) were severe. Peritoneal dialysis was required in 7 patients in the clinically severe group. As is often the case, Cr level immediately after cardiopulmonary bypass and before the initiation of peritoneal dialysis was not elevated (0.25–0.49 mg/dl). Despite the initiation of peritoneal dialysis, serum Cr increased thereafter (0.45–1.78 mg/dl) to a level meeting the criteria of acute kidney injury, defined as a >50% increase compared to the previous value.[13] With regard to changes in Cys-C and Cr before and after surgery, statistically significant increases were observed in patients <3 months of age in the clinically severe group (Figure 2). The clinically severe and non-severe groups were statistically significantly different in both Cys-C and Cr only in patients <3 months of age. To assess whether Cys-C or Cr better reflects clinical severity, the accuracy of each for identifying the clinically severe patients was analyzed based on ROC curves generated using the levels measured on the third postoperative day (Figure 2). Although the areas under the ROC curve tended to be larger for Cys-C in all age groups (Cys-C vs. Cr: <3 months of age, 0.93 vs. 0.86; 3 months–<1 year old, 0.77 vs. 0.69; ≥1 year, 0.74 vs. 0.69), the differences were not statistically significant.

To exclude the potential therapeutic bias caused by peritoneal dialysis on Cys-C and Cr level, we further performed subgroup analysis including only patients who did not undergo peritoneal dialysis. Whereas Cr level did not differ significantly between the severe and non-severe groups, Cys-C level was significantly higher in the clinically severe group than in the non-severe group in patients <3 months of age (Figure 3).

Postoperative Percent Changes in Cys-C and Cr

Figure 4 shows the relationship between the percent changes in Cys-C and Cr levels after surgery. Changes in Cys-C level generally had a good correlation with those of Cr (R=0.57, P<0.0001), but there was 1 patient who had a divergent change: the increase in Cys-C level was remarkable, as compared to that in Cr level (Figure 4; arrow). This patient had subvalvular aortic stenosis complicated by Ebstein's anomaly and surgery was performed at the age of 4 days. The patient remained critically ill postoperatively and eventually died.

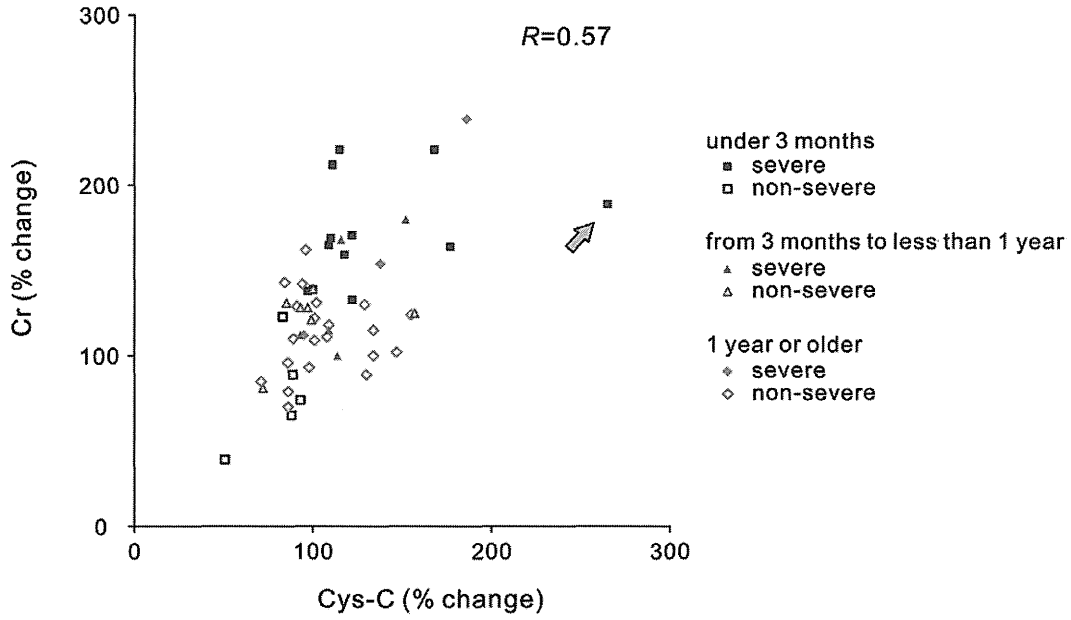

Figure 4. Relationship between pre- and postoperative changes in cystatin C (Cys-C) and creatinine (Cr) levels in pediatric patients with heart disease. There was a linear correlation between Cys-C and Cr (R=0.57, P<0.0001). Arrow, patient in whom postoperative hemodynamic status was critically unstable.

Discussion

Preoperative Cr level decreased age-dependently (in months) during the first year of life and increased age-dependently thereafter, whereas preoperative Cys-C level decreased age-dependently during the first year of life and remained almost constant thereafter regardless of age (**Figure 1**). The age-dependent changes in Cys-C and Cr were largely consistent with the results of studies on pediatric patients with disorders other than CHD.[14,16] Thus, it is suggested that GFR can be assessed using Cys-C without considering age and body size, as is necessary for Cr, even in pediatric patients with CHD, as long as the patients are >1 year of age. Because many pediatric patients with severe CHD who require evaluation are younger than 1 year of age, however, Cys-C level must be interpreted with regard to age in months.

Previous studies have suggested the potential superiority of Cys-C over Cr as a marker of renal function in several respects. Cys-C may be more sensitive to changes in GFR than Cr. Stickle et al compared Cr and Cys-C with inulin clearance, the gold standard for GFR measurement, in children aged 4–19 years. The reciprocals of Cr and Cys-C positively correlated with inulin clearance.[11] Stickle et al found that Cr does not change until inulin clearance is less than approximately 80 ml · min^{-1} · 1.73 m^{-2}.[11] In contrast, no such threshold is observed for Cys-C, and a milder decrease in inulin clearance leads to an increase in Cys-C. The difference in elevation thresholds in these indices indicates that Cys-C might detect decreased GFR earlier than Cr. This is further supported by Krawczeski et al, who examined the predictability of the onset of acute kidney injury defined as a ≥50% increase in Cr after cardiopulmonary bypass for CHD. Even when Cr was taken into consideration, Cys-C level at 12 h after surgery remained an extremely powerful predictive factor.[13] In addition, unlike Cr, serum Cys-C level is not affected by muscle mass. Cys-C can freely pass through renal glomeruli, and at least 99% of it is reabsorbed and catabolized by proximal renal tubules. Thus, Cys-C may be regarded as a better marker of GFR than Cr.

Despite the evidence suggesting the potential superiority of Cys-C over Cr as a marker of renal dysfunction, the present results failed to demonstrate that Cys-C was more sensitive for detecting clinically defined renal impairment in postoperative pediatric patients with CHD. There are several possible reasons for this observation. First, serum Cys-C was measured on the third postoperative day, when continuous diuretics or peritoneal dialysis had already been introduced. The intensive care physicians who decided whether to use diuretic drugs and to introduce peritoneal dialysis were unaware of the study design and were blinded to Cys-C level. Patient management was aimed at maintaining adequate preload and cardiac output; therefore, we cannot exclude the possibility that the results were biased by the therapeutic effects of diuretics or dialysis. Second, the definition of renal impairment in this study was based on the clinical status rather than a more robust index, such as GFR, which could have affected the results. Last, several factors have been shown to affect Cys-C level including inflammation, thyroid function, and steroid use.[3,6,7,18,19] Although none of the present patients had positive newborn screening for thyroid dysfunction, occult thyroid dysfunction, which may have accompanied cardiopulmonary bypass surgery, could have altered the results. Moreover, surgery in pediatric patients with CHD is associated with various degrees of inflammation, and steroids were given, as required, before neonatal open heart surgery or for postoperative management. Future studies with a larger number of patients should consider different factors potentially affecting Cys-C level, such as medication use including steroids. It is also of note that the present study did not provide information that is useful in

clinical decision-making, such as cut-offs for Cys-C to detect abnormal renal conditions. Because these biomarkers are expected to have a key role in clinical decision-making, future studies should define how to specifically use blood Cys-C level to evaluate children with renal impairment.

Although the limitations noted here made it difficult to draw a definitive conclusion regarding the superiority of Cys-C or Cr as a marker of renal dysfunction in the present pediatric patients, the result showing the divergent change between the 2 markers (Figure 4) may be worth noting. Moreover, subgroup analysis in patients without peritoneal dialysis (Figure 3) indicated a statistically significant difference between the severe and non-severe groups only in Cys-C. These findings may suggest a more sensitive behavior of Cys-C in the presence of severe hemodynamic disarrangement. This is another issue that merits further study.

Conclusion

Cys-C may be useful as a marker of renal dysfunction associated with hemodynamic instability, although the superiority of Cys-C over the conventional marker Cr was not confirmed in this regard. To further elucidate the clinical usefulness of Cys-C in the management of pediatric patients with heart disease, future studies with a larger number of patients are necessary and should be aimed at defining the role of Cys-C in clinical decision-making and evaluating the relationship of Cys-C with factors that may affect its levels.

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ASTUTE CLINICIAN REPORT

Clinical Course in a Patient With Neutrophil-Specific Granule Deficiency and Rapid Detection of Neutrophil Granules as a Screening Test

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Abstract
Purpose Neutrophil-specific granule deficiency (SGD) is a rare, congenital disorder characterized by atypical neutrophil structure and function that results in frequent and severe bacterial infections. However, the clinical course of patients with SGD have not been described in detail because of the scarcity of the disease. We present the clinical course of an adult patient with SGD and propose a method for making an early diagnosis of SGD.
Patient and Methods A32-year-old Japanese woman with SGD had a small impetigo lesion on her face and experienced the rapid spread of a facial abscess to a pulmonary abscess via the blood stream. We also analyzed the expression of neutrophil granule proteins in our patient compared with a healthy control by flow cytometry.
Results We confirmed defects of several neutrophil granule proteins in our patient by flow cytometry.
Conclusion Severe bacterial infections sometimes occur and spread rapidly in SGD. Detection of neutrophil granules by flow cytometry is useful for a rapid diagnosis and a screening of SGD.

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Keywords Specific granule deficiency · second granule protein · primary granule protein · flow cytometry

Introduction

Neutrophil-specific granule deficiency (SGD) is a congenital disorder characterized by atypical neutrophil structure and function that results in recurrent and severe bacterial infections such as *S. aureus* [1, 2]. Neutrophils in SGD patients exhibit a lack of several primary and secondary granule proteins, abnormalities in migration and aggregation, atypical bilobed nuclei, and impaired bactericidal activities [1–4]. Abnormalities in eosinophilic granule proteins and functional alterations of monocytes in SGD have been reported as well [5, 6]. *CEBPE*, also known as CCAAT/enhancer binding protein (C/EBP) epsilon, which is a member of the C/EBP family of transcription factors, has been implicated in SGD [7]. *CEBPE* is expressed primarily during granulocytic differentiation [7, 8]. *Cebpe* -deficient mice lack secondary granule proteins, including neutrophil gelatinase-associated lipocalin (NGAL) [9], and germ-line mutations of *CEBPE* have been detected in several SGD patients [10, 11]. A diagnosis of SGD typically involves investigation of neutrophil granule components by Western blot analysis or electron microscopy, both of which require considerable time to perform.

Clinical characteristics of SGD in adults are unclear since only a few families have been reported. In the present study, we describe the rare case of a 32-year-old female patient with SGD. We also assessed the usefulness of flow cytometric analysis of the primary granule proteins myeloperoxidase (MPO), human neutrophil peptides (HNP, defensin), and bactericidal/permeability-increasing protein (BPI), and secondary granule proteins lactoferrin, human cathelicindin

antimicrobial peptide 18 (hCAP18), and NGAL in the early diagnosis of SGD.

Patient and Methods

Case report

A 32-year-old woman had a small impetigo lesion her face that had swelled remarkably into the large cellulites and an abscess of 12 cm in maximum diameter within 1 week. She had suffered from recurrent pyogenic infections, such as otitis media, skin abscesses, and pneumonia since infancy, and we diagnosed as having SGD at the age of 7. She carries a homozygous germ-line alteration involving a single A-nucleotide insertion at nucleotide 1,113 in exon 2 of *CEBPE* [11]. Following an earlier episode of a pulmonary abscess, she had been asymptomatic doing well for more than 10 years without prophylactic therapy. On admission, the patient complained of fatigue and her body temperature was 39.5 °C. The surface of abscess had become black partially due to necrosis and she could not open her right eye due to pressure from the large abscess on her cheek (Fig. 1A). Her laboratory data were as follows: white blood cell count 29,790/mm^3 with a differential count of 69 % neutrophils, 6 % lymphocytes, 13 % monocytes, and 12 % immature granulocytes, total protein 8.3 g/dl, AST 20 U/L, ALT 18 U/L, LDH 282 U/l, IgG 2,946 mg/dl, IgA 651 mg/dl, IgM 160 mg/dl, and elevated C-reactive protein (CRP) 28.17 mg/dl. Methicillin-sensitive *S. aureus* was detected in both the facial abscess and circulating venous blood. CT imaging revealed a large mass on the right face (Fig. 1B) and a shadow indicative of pneumonia in the left lung (Fig. 1C). On the day of admission, the patient underwent the first operation for debridement of the facial abscess. She was treated with meropenem, clindamycin, and fosfomycin, and became afebrile 6 days after treatment. However, her inflammatory symptoms did not improve and her CRP levels remained high, and she subsequently experienced a recurrence of fever again on the 9th hospital day. She underwent a second operation for complete debridement of the facial abscess. Given that her circulating white blood cell count had increased to 40,470/mm^3, clindamycin was changed to vancomycin, which resulted in the gradual improvement of inflammatory symptoms and the abnormal CT findings. On the 33rd hospital day, she received a skin graft for the defect left by debridement. She has since been prophylactically treated with sulfamethoxazole-trimethoprim and had not suffered from any serious bacterial infections.

Flow Cytometric Analysis

Monoclonal antibodies (mAbs) for peridinin chlorophyll protein (PerCP)-CD45, and fluorescein isothiocyanate (FITC)-MPO, and BPI, defensin 1–3 (HNP 1–3), hCAP18, NGAL, and lactoferrin followed by FITC-gout anti-mouse IgG were purchased from BD Immunocytometry Systems (Mountain View, CA, USA), eBioscience (San Diego, CA, USA), and Hycult Biotechnology (Uden, the Netherlands), respectively. For the analysis of cytoplasmic neutrophil granules expression in peripheral blood, neutrophils were collected in polystyrene tubes and incubated with appropriately diluted PerCP-CD45, FITC-MPO or FITC-conjugated goat anti-mouse IgG or BPI, defensin, hCAP18, NGAL, or lactoferrin mAbs in the same method as described previously [6]. The cells were washed twice and analyzed with a FACScan flow cytometer and the Lysis II software (BD Immunocytometry Systems). Viable cells were gated according to their forward-scatter and side-scatter characteristics.

Result

We analyzed the expression of primary and secondary granule proteins in our SGD patient and in a healthy normal control by flow cytometry. Control neutrophils contained abundant primary and secondary granule proteins. In contrast, the patient exhibited markedly reduced levels of the primary granule defensins (Fig. 2A) and secondary granules lactoferrin, NGAL, and hCAP18 (Fig. 2B). BPI expression was slightly reduced in comparison with the control. Meanwhile, MPO was expressed at similar levels on neutrophils from both subjects.

Discussion

The clinical course of SGD patients has not been well described because of the scarcity of the disease. We described

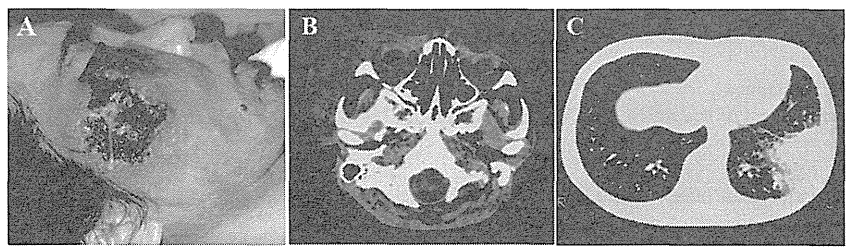

Fig. 1 Imaging findings of impetigo lesion on face and lung. Photograph of facial abscess A, and computed tomography imagings of face B and chest C

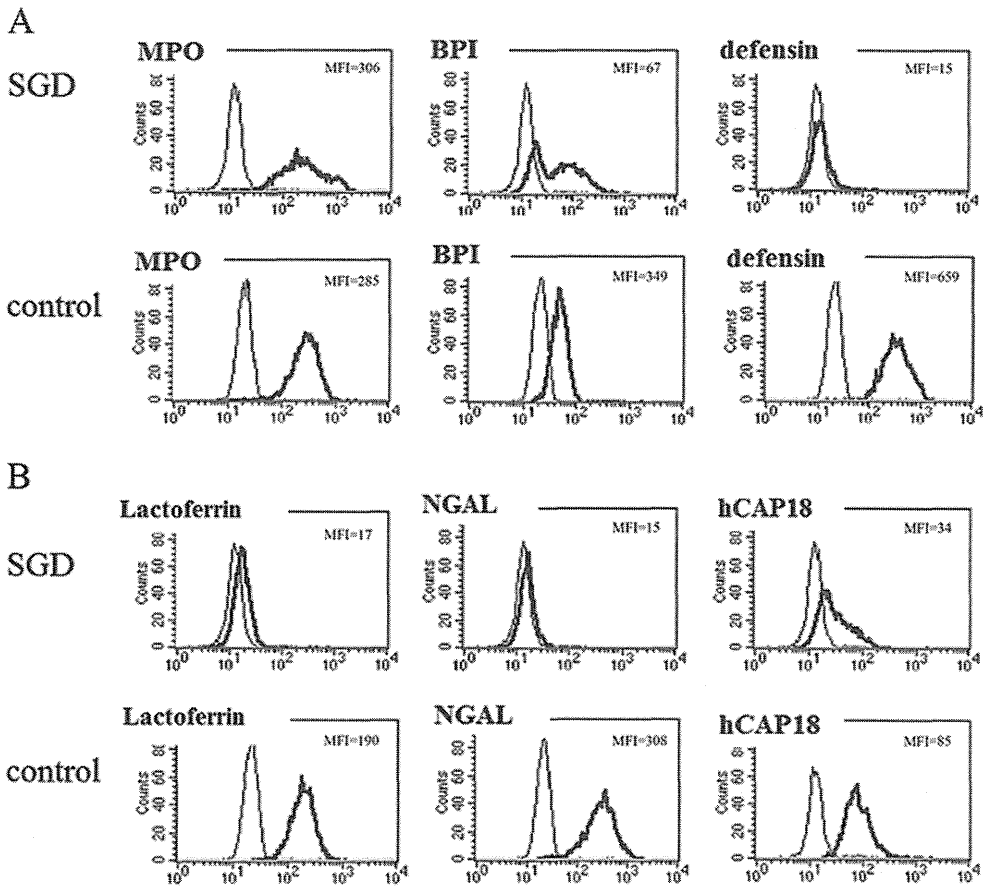

Fig. 2 Expression of MPO, BPI, defensin, lactoferrin, NGAL, and hCAP18 in neutrophils from SGD patient and control. Peripheral blood cells from our SGD patient and tree healthy individuals (controls) were fixed, permeabilized, and stained for primary granules (A; MPO, BPI, and defensin) and secondary granules (B; lactoferrin, NGAL, and hCAP18). The results of a flow cytometric analysis are shown as histograms and mean fluorescent intensities (MFI). MFI of each granule from controls (mean±SD, n=3) were as follows; MPO 303±61, BPI 353 ±12, defensin 892±265, lactoferrin 199±63, NGAL 272±41, and hCAP18 68±16

the clinical course of a patient with SGD who experienced the rapid spread of a facial abscess to a pulmonary abscess via the blood. We also evaluated a method to make an early diagnosis of SGD by detecting neutrophil granule proteins with flow cytometry. The aggressive clinical course seen in our patient indicated that recurring infections progress rapidly and become more severe in SGD. Thus, prophylactic use of antibiotics, such as sulfamethoxazole-trimethoprim, may be important to prevent further bacterial infections.

Neutrophils play an important role in innate immune responses against microorganisms. Neutrophil abnormalities of migration, phagocytosis, bactericidal activity have been reported in mice models [12]. In contrast, in human, migration abnormality was reported by Strauss et al.[1]. Also, bactericidal activity was abnormal, but normal about phagocytosis [1, 3] Bactericidal activity and chemotaxis decrease in our patient [2], but we were not able to evaluate the chemotaxis exactly afterword because of technical problem. During phagocytosis, neutrophils release a large amount of reactive oxygen species (ROS) through NADPH oxidase system. Neutrophils also possess bactericidal proteins packaged in primary and secondary granules [13]. Neutrophils in SGD patients lack several secondary granule proteins, including lactoferrin, and hCAP18. The expression of the primary granules defensin and BPI are nearly absent as well [11, 14], but MPO and lysozyme expression is unaffected [14]. Although the neutrophils of SGD patients produce normal levels of ROS, the frequency and severity of infections are similar to those in chronic granulomatous disease, which exhibit defective ROS production. This indicates that both granule proteins and ROS play critical roles in neutrophils in neutrophil-mediated bactericidal activities.

A diagnosis of SGD was already made on the basis of clinical characteristics and cell morphology in our patient. We here show that detection of neutrophil granules by flow cytometry is useful for screening and conforming diagnosis of SGD.

Similarly to conventional analyses such as responsible gene analysis, we confirmed deficiencies in granule proteins in SGD neutrophils by flow cytometry. Flow cytometry is useful for screening neutrophil granule content because samples can be prepared simply and quickly for analysis, and several proteins can be analyzed at once. Given that the gene analysis is needed time and an expense, when we consider SGD by clinical and laboratory findings, it is desirable to detect the granule proteins at first, and to perform gene analysis afterwards. Although a mutation in the *CEBPE* gene was responsible for SGD in our patient, this mutation has not been

detected in other cases [15]. Thus, factors apart from *CEBPE* may be associated with disease etiology. We believe that our simple flow cytometric-based technique will be useful not only to rapidly assess neutrophil granules and to be adopted as a screening test for SGD, but also to find out new responsible gene in patients with susceptibilities to infections due to neutrophil dysfunction.

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Conflict of interest The authors declare no conflicts of interest.

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of the present patient refused additional treatment. The present patient received no treatment for the parotid lymphangioma after the open biopsy and prednisolone treatment. Facial nerve paralysis was completely cured 1 month after medication. The left parotid lymphangioma has remained unchanged and there has been no recurrent facial nerve paralysis for 8 years.

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Peripheral blood flow monitoring in an infant with septic shock

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Abstract Septic shock is associated with impaired vasoregulation, and treatment includes vasoactive drugs. Therefore, evaluation of vasoregulatory change is important. The present report describes the successful characterization of vasoregulatory change in response to a vasoactive drug during septic shock. A male infant born at 23 weeks' gestation developed septic shock. Severe hypotension developed, and treatment with colloid fluid and dopamine failed to increase blood pressure. With continuous measurement of skin blood flow using laser Doppler, noradrenaline was started. Based on changes in the blood flow, the dose was increased. At a dose of 1 μg/kg per min, skin blood flow in the foot decreased without any change in blood pressure. Subsequent blood transfusion succeeded in increasing both blood pressure and skin blood flow. It is concluded that decrease in foot blood flow reflects the vasoconstrictive effect of noradrenaline, although this finding must be validated in larger studies.

Key words laser Doppler flowmetry, microcirculation, regional blood flow, sepsis, septic shock.

In infants with septic shock, impaired peripheral vasoregulation can cause circulatory changes. These infants present with either warm shock caused by vasodilation or cold shock caused by vasoconstriction.[1] Because vasoregulatory changes can appear before specific evidence of the apparent shock, the detection of these vasoregulatory changes would facilitate early intervention for septic shock. In addition, because the circulatory support for such conditions usually consists of vasoactive drugs, an accurate method to monitor the vasoconstrictive or vasodilative effects of the drugs would be of benefit. Direct measurement of skin blood flow using a laser Doppler flowmeter[2,3] can detect early changes in peripheral circulation. We previously described the effect of dopamine on peripheral circulation in very low-birthweight infants.[2] We suspected that the method would be useful for the detection of the effects of the vasoactive drugs in infants with septic shock.

This case report describes the successful detection of changes in skin blood flow in an extremely low-birthweight infant who was successfully treated for septic shock.

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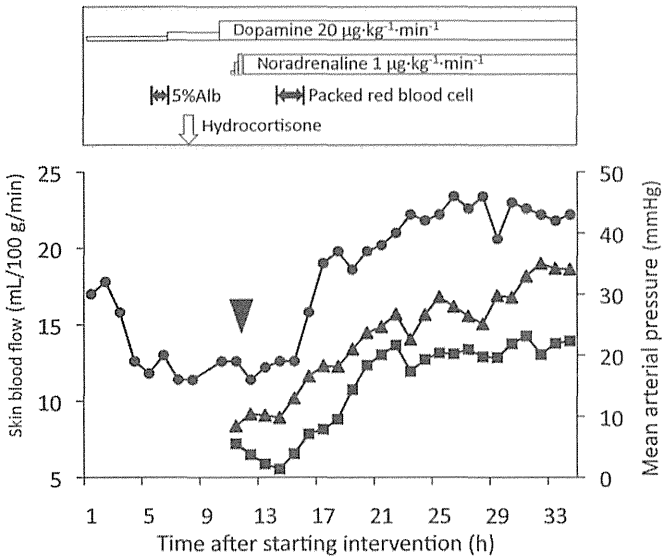

Fig. 1 Changes in (●) mean blood pressure and in skin blood flow in the (■) foot and (▲) forehead. Skin blood flow at each point was calculated by averaging over 1 h. Data points were obtained every 10 s. When noradrenaline reached 1 μg/kg/min, the skin blood flow in foot started to decrease (arrowhead).

Case report

A male infant was born at 23 weeks 5 days of gestational age by emergency cesarean delivery for non-reassuring fetal status. Birthweight was 601 g. He was intubated immediately after birth and underwent mechanical ventilation due to respiratory distress syndrome. On postnatal day 8, the infant became less active, SpO_2 gradually decreased, and the infant required higher fraction of inspiratory oxygen. A few hours after the deterioration of respiratory status, mean arterial blood pressure (MAP) decreased from 30 mmHg to 25 mmHg over a few hours. Laboratory tests indicated leukopenia, thrombocytopenia, and marked elevation of C-reactive protein and blood glucose. Respiratory acidosis was also present. On chest X-ray a right upper infiltrative shadow was seen. From these findings, the infant was clinically diagnosed with pneumonia and septic shock. Antibiotics were given, dopamine was started at 4 μg/kg per min, and 10 mL/kg of 5% albumin was given for 1 h to treat hypotension. The initial dose of dopamine failed to increase MAP sufficiently and, 6 h later, MAP decreased to 15 mmHg. Therefore, 20 mg/kg hydrocortisone was given, and the dose of dopamine was increased to 20 μg/kg per min, but this intervention failed to increase MAP. Echocardiography showed superior vena cava blood flow of 78 mL/kg per min and an ejection fraction of 89.5%. These findings indicated good left ventricle function and relatively maintained systemic blood flow, and we presumed that hypotension was not due to vasoconstriction despite the fact that the physical findings (e.g. cold extremities) at that time indicated cold shock. With continuous monitoring of skin blood flow at the forehead and foot using a laser Doppler flowmeter (CDF-2000; Nexis, Fukuoka, Japan),[2,3] noradrenaline infusion was started at a dose of 0.2 μg/kg per min. Because there was no change in blood pressure or in skin blood flow, we increased the dose of noradrenaline to 1 μg/kg per min within 1 h. When the dose reached 1 μg/kg per min, skin blood flow in the foot decreased from 7.2 mL/100 g tissue weight per min to 5.5 mL/100 g tissue weight per min without a significant change in MAP (Fig. 1). Next, 10 mL/kg packed red blood cell transfusion over 2 h was given as a volume expander. After starting the transfusion, MAP and skin blood flow in the foot and forehead markedly increased.

Written, informed consent was obtained from the infants' parents. This study was approved by the Ethics Committee of Saitama Medical Center, Saitama, Japan.

Discussion

In this study, continuous monitoring of skin blood flow enabled characterization of the effects of vasoactive drugs in an infant with septic shock. In 2007, the American College of Critical Care Medicine proposed guidelines for the hemodynamic support of pediatric and neonatal patients with septic shock.[4] The recommended initial step was a fluid bolus, and dopamine was usually selected as a first-line vasoactive drug. In the present case, both 5% albumin infusion and dopamine at a dose up to 20 μg/kg per min failed to increase blood pressure.

For volume- and inotrope-refractory hypotension, use of noradrenaline or adrenaline is recommended. In the guidelines, the use of noradrenaline for hypotension with warm shock and the use of adrenaline for hypotension with cold shock is recommended.[4] The distinction between cold and warm shock is made by various physical findings, including capillary refill time, peripheral pulses, cold or warm extremities, and urine output, all of which depend on the amount of peripheral blood flow. A previous study reported that warm shock was an uncommon condition[5,6] or at least was difficult to recognize in neonates.[7] The guidelines recommend the use of low-dose adrenaline only after dopamine, which can cause vasodilation. In the present case, the skin blood flow in the foot before noradrenaline was 7.2 mL/100 g tissue weight per min, and it was lower when compared with previous studies (19.1 ± 4.5 mL/100 g tissue weight per min at 48 h after birth in very low-birthweight infants),[3] which might have caused cold extremities and prolonged capillary refill time. Echocardiography, however, showed good left ventricular function and sustained systemic blood flow during severe hypotension. Considering the formula, blood pressure = systemic blood flow × vascular resistance, severe hypotension with sustained systemic blood was not consistent with high vascular resistance. Indeed, factors other than vasoconstriction can decrease skin blood flow in the foot, such as low peripheral perfusion pressure subsequent to severe hypotension and relative hypovolemia. Thus, we speculated that we should not limit the use of vasoconstrictors based on physical findings alone that seemed to indicate the presence of cold shock. In the present case, noradrenaline was chosen to induce vasoconstriction. At a dose of 1 μg/kg per min, skin blood flow in the foot significantly decreased without any change in MAP; these changes were considered to be induced by the vasoconstrictive effect of the drug. Subsequent packed red blood cell transfusion succeeded in increasing both blood pressure and skin blood flow. The reason why noradrenaline did not

increase blood pressure was unclear but, based on the ability of packed red blood cells to increase MAP and skin blood flow, it was suspected that relative hypovolemia prevented noradrenaline from increasing blood pressure. The apparent difference between the effects of volume expanders (i.e. 5% albumin and packed red blood cells) is explained by differences in the stability of these products within the blood vessels. Another possible explanation, however, is that vasoconstriction prior to the use of volume expander supported the effect of the volume expander by reducing the volume of the peripheral vascular bed and/or by decreasing capillary leakage. All of these observations must be confirmed by simultaneously measuring systemic blood flow, blood pressure and skin blood flow within larger studies.

Although an accurate method of determining the effect of vasoactive drugs on the peripheral circulation in real time would be invaluable, no method or marker has previously been established to detect the changes in vasoregulation in neonates. Cardiac index and systemic vascular index (which are calculated from the MAP and cardiac output) have been previously used to evaluate abnormal vasoregulatory changes in children with septic shock.[8] In neonates, however, shunt through the patent ductus arteriosus and foramen ovale makes it difficult to appropriately measure cardiac output and to calculate systemic vascular resistance. Capillary refill time also plays a role in the management of the septic shock,[4] but this parameter is likely insufficient for detection of slight changes in peripheral blood flow. Recently developed orthogonal polarization spectral imaging techniques enable direct measurement of vessel diameter and functional capillary density,[9,10] and can be used to assess vasoregulatory changes in neonates with septic shock. That method, however, is not suitable for prolonged and continuous monitoring. In contrast, continuous and real-time measurement of the skin blood flow using laser Doppler apparatus can detect the vasoregulatory changes induced by noradrenaline and facilitate determination of the optimal therapeutic dose of the drug. No previous study has shown direct evidence of the effects of vasoactive drugs, and we assume that studies using this technique in large subject groups would help to elucidate the mechanism of action of vasoactive drugs.

Acknowledgments

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Case of early childhood-onset narcolepsy with cataplexy: Comparison with a monozygotic co-twin

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Abstract We describe here a rare case of early childhood-onset (5 years of age) narcolepsy. This case was interesting because of the ability to compare the patient's symptoms to the condition of her healthy monozygotic co-twin sister. The only environmental difference between the co-twins was head injury, which may be associated with the presence of

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Skin blood flow as a predictor of intraventricular hemorrhage in very-low-birth-weight infants

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BACKGROUND: Cardiovascular instability immediately after birth is associated with intraventricular hemorrhage (IVH) in very-low-birth-weight (VLBW) infants. For circulatory management, evaluation of organ blood flow is important. In this study, the relationship between peripheral perfusion within 48 h after birth and IVH was evaluated in VLBW infants.

METHODS: In this prospective observational study involving 83 VLBW infants, forehead blood flow (FBF) and lower-limb blood flow (LBF) were measured for 48 h after birth using a laser Doppler flowmeter. Blood flow was compared between infants with and without IVH. Multivariate logistic regression analysis was performed to identify the risk factors for IVH.

RESULTS: IVH developed in nine infants. In eight of these patients, IVH occurred after 24 h. LBF was lower in infants with IVH at 18 and 24 h and increased to the same level as that of infants without IVH at 48 h. Multivariate logistic regression analysis identified a correlation only between LBF and IVH at 18 h.

CONCLUSION: These findings were consistent with the hypoperfusion–reperfusion theory, which states that IVH develops after reperfusion subsequent to hypoperfusion. We speculate that measurement of skin blood flow in addition to systemic and cerebral circulation may be helpful in predicting IVH.

Intraventricular hemorrhage (IVH) is one of the major causes of long-term neurological disability in very-low-birth-weight (VLBW) infants. In almost 90% cases, IVH occurs in the first 4 d of life (1). Several studies found an association between cardiovascular instability immediately after birth and IVH in VLBW infants (2–6).

Circulatory management in the neonatal intensive care unit (NICU) mainly focuses on elevation of blood pressure (6,7). Several studies have identified an association between hypotension and poor neurodevelopmental outcomes (1,3). However, no data from randomized controlled trials have been available to support the assumption that treatment of hypotension has a beneficial effect on mortality rates or IVH incidence in preterm infants (9–11). The failure of this management strategy in improving prognosis may be related to the fact that improvement of perfusion pressure by elevation of blood pressure does not always correspond to an adequate increase in organ blood flow, although the ultimate goal of circulatory management is to maintain organ perfusion and O_2 delivery.

The importance of evaluating systemic blood flow and blood flow to all organs is being increasingly recognized (12). Some recent studies have demonstrated correlations between poor outcomes and low blood flow or hypoperfusion of both vital and nonvital organs in preterm infants (13–16). Regarding peripheral perfusion, Dempsey *et al.* (17) suggested a correlation between poor outcome and clinical signs of peripheral hypoperfusion such as urinary output, capillary refill time, and skin color during the early neonatal period in preterm infants. Assessment of peripheral perfusion is an important part of the clinical exam, as impairment of peripheral perfusion is one of the early markers of the compensated phase of neonatal cardiovascular compromise. However, capillary refill time, the most frequently used parameter to assess peripheral perfusion in the clinical practice, is a poor predictor of low systemic blood flow. Therefore, use of a more objective, real-time, noninvasive marker of peripheral perfusion would be preferred, at least in the research setting.

In this study, the correlation between IVH following postnatal circulatory instability during the extrauterine transitional period and skin and subcutaneous blood flow was investigated. Blood flow measurements were performed using a laser Doppler flowmeter in VLBW infants treated for hypotension during the first 48 h after birth.

RESULTS

In total, 83 infants were included in this study. Head ultrasonography confirmed that no IVH had developed in 74 infants at 1–3, 6, 24, and 48 h after birth and on postnatal days 4 and 7. Head ultrasonography was performed at least once a day in all infants with IVH. Head ultrasonography confirmed IVH in nine infants at 24 h (n = 1), between 24 and 48 h (n = 4), between 48 and 72 h (n = 3), and on day 6 (n = 1). Among these, five infants progressed to severe IVH. In all cases of severe IVH, progression of IVH from grade 2 to 3 or 4 occurred after 48 h (n = 2 on day 3, n = 2 on day 4, n = 1 on day 6). Infants

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Table 1. Demographic characteristics of the infants

Characteristic	IVH	No IVH	*P* value
n	9	74	
Gestational age (weeks)	26.0 (23.4–27.7)	27.6 (23.3–34.3)	0.009
Birth weight (grams)	726 (416–1186)	805 (374–1478)	0.55
Female	3 (33%)	45 (62%)	0.15
Preeclampsia	1 (11%)	12 (16%)	1.0
Antenatal steroid	3 (33%)	50 (68%)	0.07
Cesarean section	8 (89%)	69 (93%)	1.0
Ventilated	8 (89%)	59 (80%)	0.68
Apgar score[a]			
1 min	4 (3–5)	5 (3–7)	0.11
5 min	7 (6,7)	8 (6–9)	0.04
Dopamine administration	9 (100%)	69 (81%)	0.35
Maximum dose of dopamine	6.2 (4.9–21.0)	4.8 (0–20.7)	0.02
Volume expander administration	2 (22%)	6 (8%)	0.21

Values are expressed as number (%) or median (range) unless otherwise indicated.

IVH, intraventricular hemorrhage.

[a]Values are expressed as median (25%–75% quartiles).

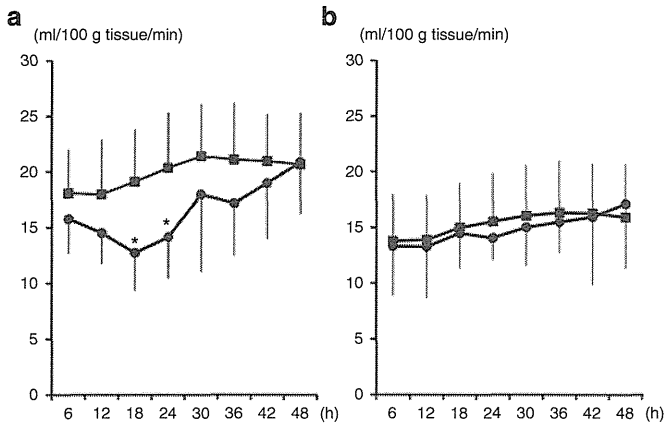

Figure 1. Temporal changes in skin and subcutaneous blood flow. (**a**) Lower-limb blood flow (LBF) in infants with and without intraventricular hemorrhage (IVH). (**b**) Forehead blood flow (FBF) in infants with and without IVH. Filled squares, without IVH ($n = 74$); filled circles, with IVH ($n = 9$). Due to infant death ($n = 1$) and due to temporary removal and displacement of the blood flow probe for interventions, some data were lost. The values were therefore calculated from available data (LBF, $n = 72$, $n = 71$, and $n = 71$ at 30, 36, and 42 h, respectively, in infants without IVH; $n = 8$, $n = 7$, $n = 7$, $n = 7$, and $n = 8$ at 18, 30, 36, 42, and 48 h, respectively, in infants with IVH; FBF, $n = 71$, $n = 70$, $n = 69$, $n = 70$, $n = 69$, $n = 66$, $n = 67$, and $n = 69$ at 6, 12, 18, 24, 30, 36, 42, and 48 h, respectively, in infants without IVH; $n = 8$, $n = 7$, $n = 7$, $n = 7$, and $n = 8$ at 18, 30, 36, 42, and 48 h, respectively, in infants with IVH). Differences between groups, $^*P < 0.006$ (Bonferroni correction *P*-value criteria < 0.006).

with IVH were born at earlier gestational age, had lower 5-min Apgar scores, and received higher dopamine doses (Table 1).

No significant changes were made in the dopamine dose administered to infants with and without IVH. Mean values (SD) in $\mu g \cdot kg^{-1} \cdot min^{-1}$ were as follows: 9.0 (7.4), 10.7 (7.5), and 8.2 (6.2) at 6, 24, and 48 h, respectively; and 4.2 (3.4), 5.0 (4.4), and 4.5 (4.1) at 6, 24, and 48 h, respectively. Epinephrine was administered to three of the nine (33%) infants with IVH; in two of these, it was initiated between 24 and 48 h after birth.

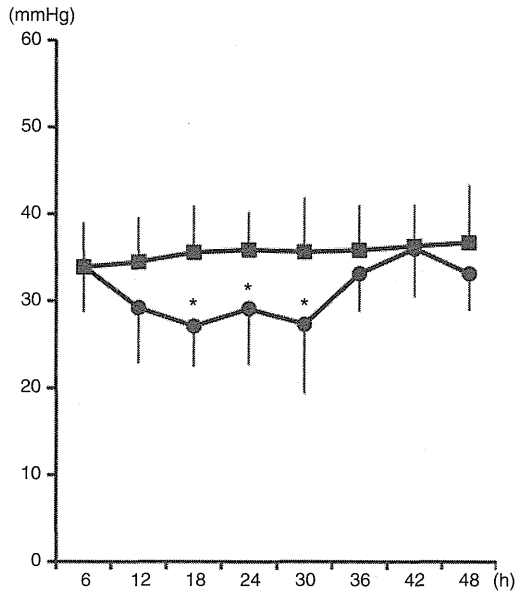

Figure 2. Temporal changes in the mean arterial blood pressure. Differences between groups, $^*P < 0.006$ (Bonferroni correction *P*-value criteria < 0.006). Filled squares, without intraventricular hemorrhage (IVH) ($n = 74$); filled circles, with IVH ($n = 9$). Due to temporary removal and displacement of the blood flow probe for interventions and due to discontinuity by death, some data were lost. Values were therefore calculated from available data ($n = 72$, $n = 73$ at 36 and 42 h, respectively, in infants without IVH; $n = 8$, $n = 8$, and $n = 8$ at 36, 42, and 48 h, respectively, in infants with IVH).

Differences in Lower-Limb Blood Flow (LBF), Forehead Blood Flow (FBF), and Mean Arterial Blood Pressure (MAP) Between Infants With and Without IVH

The mean LBF at 18 and 24 h was lower in infants with IVH than in those without (18 h: 12.7 (3.4) ml/100 g and 19.1 (4.7) ml/100 g tissue weight/min, $P = 0.0004$; 24 h: 14.2 (3.7) ml/100 g and 20.4 (5.0) ml/100 g tissue weight/min, $P = 0.0005$; Bonferroni correction *P*-value criteria <0.05/8 = 0.006; Figure 1a). No difference in FBF was found between the groups at any time point (Figure 1b).

MAP was lower in infants with IVH than in those without IVH at 18, 24, and 30 h (18 h: 27 (5) mmHg vs. 36 (5) mmHg, $P < 0.001$; 24 h: 31 (5) mmHg vs. 36 (5) mmHg, $P = 0.004$; 30 h: 27 (8) mmHg vs. 36 (6) mmHg, $P < 0.001$; Bonferroni correction *P*-value criteria <0.05/8 = 0.006; Figure 2).

Temporal Changes in LBF, FBF, and MAP

In infants with IVH, LBF decreased until 18 h, following which it gradually increased until 48 h. At 42 h, this increase was statistically significant (18 vs. 42 h, $P < 0.05$; Figure 1a). In infants without IVH, no change in LBF was observed until 12 h. Thereafter, LBF gradually increased until 30 h. At 24 h, the increase was statistically significant (6 vs. 24 h, $P < 0.01$; 12 vs. 24 h, $P < 0.01$; Figure 1a). After 30 h, LBF remained unchanged until 48 h.

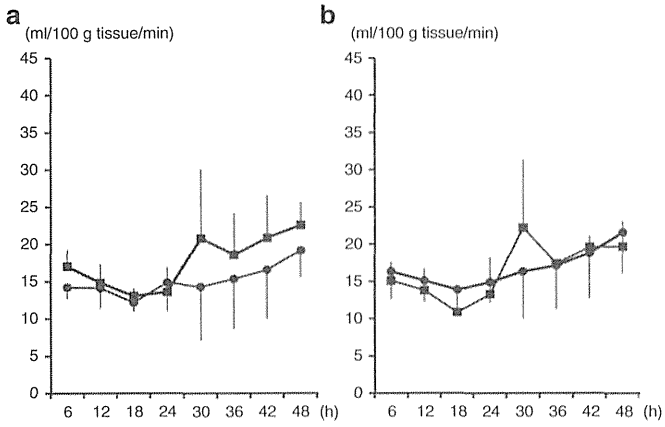

Figure 3. Lower-limb blood flow (LBF) and the timing of development and severity of intraventricular hemorrhage (IVH). (**a**) LBF in infants with IVH developing within 48 h and after 48 h. Filled squares, IVH developing within 48 h ($n = 5$); filled circles, IVH developing after 48 h ($n = 4$). (**b**) LBF in infants with grade 2 IVH and grade 3 or 4 IVH. Filled squares, grade 2 IVH ($n = 4$); filled circles, grade 3 or 4 IVH ($n = 5$).

No significant temporal change in FBF was observed in infants with IVH. In infants without IVH, no change in FBF was observed until 12 h. Thereafter, FBF gradually increased until 36 h. At 24 h, this increase was statistically significant (6 vs. 24 h, $P < 0.05$; Figure 1b), following which FBF remained unchanged until 48 h.

In infants with IVH, MAP gradually decreased until 18 h and then remained unchanged until 30 h. After 30 h, MAP gradually increased until 42 h (12 vs. 42 h, $P < 0.05$; 18 vs. 42 h, $P < 0.01$; 30 vs. 42 h, $P < 0.05$; Figure 2). In infants without IVH, MAP gradually increased until 48 h. At 42 h, this increase was statistically significant (6 vs. 42 h, $P < 0.05$; Figure 2).

LBF and the Timing of Development and Severity of IVH

No difference in LBF was detected at any time point between infants in whom IVH developed within 48 h and those in whom IVH developed between 48 h and day 7 (Figure 3a). No difference in LBF was found between infants with grade 2 IVH and infants with grade 3 or 4 IVH at any time point (Figure 3b).

Risk Factors for IVH

Univariate regression analysis was performed for the 12 demographic and clinical factors shown in Table 1 as well as MAP, FBF, and LBF at 6, 12, 18, and 24 h. Univariate logistic regression analysis revealed that earlier gestational age, no antenatal steroid administration, higher maximum dose of dopamine, lower MAP at 12 and 18 h, and lower LBF at 18 and 24 h were significant risk factors for IVH (Table 2). Multivariate regression analysis was performed at 18 h, the lowest point for LBF and MAP during the study period. For the analysis, MAP at 18 h, FBF at 18 h, LBF at 18 h, and factors in Table 2 with P values of < 0.15 between groups were selected. Multivariate logistic regression models revealed only LBF at 18 h as a significant risk factor (Table 2).

The receiver operator curves for MAP, FBF, and LBF at 18 h were determined. The area under the curve best cut-off value, sensitivity, specificity, and positive and negative predictive values are shown in Table 3. Significantly higher area under the curve values were observed for LBF than for FBF ($P = 0.001$), but not for MAP ($P = 0.92$).

Table 2. Univariate and multivariate odds ratio for IVH

Variable	Univariate OR (95% CI)	Adjusted OR (95% CI)
GA[a]	0.59 (0.38–0.93); $P = 0.02$	1.95 (0.53–7.23)
Female sex	0.39 (0.09–1.74)	0.22 (0.009–5.60)
Antenatal steroids	0.16 (0.03–0.85); $P = 0.03$	0.13 (0.004–4.50)
Apgar score		
1 min	0.83 (0.59–1.17)	0.32 (0.06–1.56)
5 min	0.82 (0.56–1.20)	1.03 (0.25–4.3)
Maximum dose of dopamine[b]	1.13 (1.00–1.28); $P = 0.04$	1.08 (0.81–1.44)
MAP at 18 h[c]	0.69 (0.57–0.85); $P < 0.001$	0.69 (0.41–1.17)
LBF at 18 h[d]	0.58 (0.39–0.84); $P = 0.005$	0.38 (0.14–0.99); $P = 0.049$
FBF at 18 h[d]	0.97 (0.80–1.17)	1.24 (0.74–2.06)

Eight out of nine infants in the IVH group who developed IVH after 24 h were included in this analysis.

CI, confidence interval; FBF, forehead blood flow; IVH, intraventricular hemorrhage; LBF, lower-limb blood flow; MAP, mean arterial blood pressure; OR, odds ratio.

[a]OR for each additional week. [b]OR for each 1 μg·kg−1·min−1 increment. [c]OR for each 1 mmHg increment. [d]OR for each 1 ml/100g tissue weight/min increment.

Table 3. MAP, LBF, and FBF at 18 h as predictors for IVH. ROC curve

Value	AUC	SE	Cutoff	Sensitivity	Specificity	PPV	NPV
MAP	0.89	0.05	32	0.88	0.78	0.33	0.98
LBF	0.89	0.08	13.8	0.88	0.93	0.58	0.99
FBF	0.50	0.09	16.0	0.75	0.41	0.13	0.93

In total, 8/9 infants in the IVH group who developed IVH after 24 h were included in this analysis.

AUC, area under the curve; FBF, forehead blood flow; IVH, intraventricular hemorrhage; LBF, lower-limb blood flow; MAP, mean arterial blood pressure; NPV, negative predictive value; PPV, positive predictive value; ROC, receiver operating characteristic.

DISCUSSION

The major finding of this study was that compared to infants without IVH, lower LBF values were observed until 24 h after birth in infants who developed IVH between 6 h and 7 d after birth. After 24 h, these values increased to a level similar to (but not exceeding) those in infants without IVH at 48 h. FBF and MAP also showed increasing trends over time in infants with IVH. Trends of FBF in infants with IVH were not statistically significant, probably because of the relatively small number of infants with IVH. Considering that IVH developed after 24 h in all cases but one, we conclude that IVH developed following a period of low blood flow and during a period of increased blood flow to a level not exceeding those in non-IVH infants. These findings are consistent with the hypoperfusion–reperfusion theory on the development of IVH, which states that IVH develops after reperfusion subsequent to hypoperfusion (18,19). Lack of data after 48 h precluded evaluation of additional changes in blood flow after 48 h in infants in whom IVH developed after this time point. Causes of progression to

severe IVH after 48 h were therefore unclear. However, low blood flow within 24 h and subsequent recovery of blood flow were observed in infants in whom IVH developed after 48 h and in infants in whom IVH progressed in severity. Assessment of peripheral blood flow and systemic blood pressure might identify very preterm neonates at higher risk for IVH during the transitional period.

The reasons behind the correlation between LBF and IVH and the lack of correlation between FBF and IVH are unclear. We previously reported a positive but weak correlation between LBF and superior vena cava blood flow (20). Superior vena cava blood flow has been used as a marker of upper-body systemic blood flow, including venous return from the brain (21). Previous studies reported an association between low superior vena cava blood flow within the first 48 h of life and IVH (4,22). However, the extent to which superior vena cava blood flow accounts for cerebral blood flow in VLBW infants remains unclear. Cerebral blood flow is different from systemic and peripheral blood flow because it is regulated by several brain-specific factors such as cerebral autoregulation and flow–metabolism coupling. Correlations among systemic, cerebral, and peripheral blood flow must be investigated by simultaneous measurement of these parameters for better understanding of the mechanism underlying IVH development.

Previous research has suggested that because low cerebral blood flow and low blood pressure progress toward an ischemic threshold, the structural integrity of brain tissue is gradually compromised (23). Subsequent increase in blood flow may then cause bleeding from the destroyed vessels. On the basis of the data in the literature and the present findings, future interventional studies in very preterm infants might target preventing systemic and brain hypoperfusion immediately after delivery or, if hypoperfusion is not preventable, controlling the reperfusion rate of reperfusion for potential improvement of short- and long-term neurodevelopment.

Although several factors (including MAP) differed between the infants with and without IVH, after adjusting for these factors, only LBF correlated with IVH. The main goal of circulatory management in the NICU, especially for preterm infants, has been to maintain blood pressure using various therapeutic interventions. Several numerical standards of blood pressure have been suggested (24), and MAP has been a frequently used parameter in clinical settings. However, no previous studies have shown a decreased IVH incidence by management of blood pressure according to any standard. Therefore, this study was conducted to address this issue.

Blood pressure–related intervention was also provided to all infants participating in this study. Although MAP was lower in infants with IVH than in those without, MAP within the target range was achieved until the 24-h time point among all infants with IVH except one. Along with the data in the literature, the results of this study suggest that for the development of more effective treatment strategies to prevent IVH, parameters of blood flow along with blood pressure measurement need to be monitored and that this approach may include skin and subcutaneous blood flow measurement.

Findings of the present study also suggest that skin and subcutaneous blood flow might be a predictor of IVH. FBF was a less effective predictor of IVH than LBF. In our previous investigation on the hemodynamic effects of dopamine in VLBW infants (25), FBF was less informative than changes in LBF. No clear explanation for this finding exists at present; however, it may have been caused by differences in autonomic regulation between these two regions. The skin of the lower limb is innervated by sympathetic nerves alone, while blood flow in the facial skin is regulated by both sympathetic and parasympathetic nerves, such as the blood flow in the internal vital organs (26).

Frequent use of vasopressor-inotropes was a major limitation in this study. Dopamine was administered in the majority of infants in both groups, and the maximum dose was higher in infants with IVH than in those without. However, doses did not change significantly over time in either group. Concerns have been raised about the potential vasoconstrictive affects of dopamine (27). However, the results of our previous study (25) demonstrated that even if the dose was 10–20 μg/kg/min, dopamine significantly increased LBF in VLBW infants. Cardiovascular adrenergic receptor downregulation might explain the decreased cardiovascular responsiveness to dopamine in critically ill preterm infants (28). Therefore, the low blood flow in infants with IVH within 24 h was not considered to have resulted from a vasoconstrictive effect of dopamine. Epinephrine, which is considered to have a stronger vasoconstrictive effect than that of dopamine, was administered to three infants with IVH and may have decreased LBF. However, administration of this drug was initiated after 24 h in two of the three cases, and LBF was already below the cutoff value before administration in the other case. Therefore, epinephrine use in the present study may have had only a minimal effect on low LBF values in the infants with IVH within 24 h.

In conclusion, the findings of this observational study suggest that decreased peripheral perfusion may predict the subsequent development of IVH in VLBW infants. These findings lend support to the hypothesis that hypoperfusion–reperfusion cycle plays a role in the pathogenesis of IVH. Larger studies using additional measures of brain (such as near-infrared spectroscopy) and systemic (echocardiography, electrical impedance) blood flow with LBF and FBF measurements are required to further clarify these findings.

METHODS

Written informed consent was obtained from the infants' parents immediately on admission to the NICU. This study was approved by the Ethical Committee of Saitama Medical Center, Saitama, Japan.

Subjects

This was a prospective observational study of infants born with a body weight of <1,500 g at birth. Three hundred and eighty-five VLBW infants were born in the period between 1 September 2008 and 30 September 2012 and admitted to the NICU at Saitama Medical Center. To avoid interobserver error, the study was initiated when one specific investigator (A.I.) and the laser Doppler apparatus were both available (n = 111). Exclusion criteria included compromise other than prematurity, which could affect hemodynamics of the infants: congenital heart disease (n = 7), chromosomal and/or major