ASSOCIATION BETWEEN SEX, NUTRITIONAL STATUS, SEVERITY OF DENGUE HEMORRHAGIC FEVER, AND IMMUNE STATUS IN INFANTS WITH DENGUE HEMORRHAGIC FEVER

NGUYEN THANH HUNG, NGUYEN TRONG LAN, HUAN-YAO LEI, YEE-SHIN LIN, LE BICH LIEN, KAO-JEAN HUANG, CHIOU-FENG LIN, DO QUANG HA, VU THI QUE HUONG, LAM THI MY, TRAI-MING YEH, JYH-HSIUNG HUANG, CHING-CHUAN LIU, AND SCOTT B. HALSTEAD

Department of Dengue Hemorrhagic Fever, Children’s Hospital No. 1, Ho Chi Minh City, Vietnam; Department of Microbiology and Immunology, Department of Medical Technology, and Department of Pediatrics, College of Medicine, National Cheng Kung University, Tainan, Taiwan, Republic of China; Arbovirus Laboratory, Pasteur Institute, Ho Chi Minh City, Vietnam; Department of Pediatrics, University of Medicine and Pharmacy, Ho Chi Minh City, Vietnam; Division of Research and Diagnosis, Center for Disease Control, Department of Health, Taipei, Taiwan, Republic of China; Uniformed Services University of the Health Sciences, Bethesda, Maryland

Abstract. The association between sex, nutritional status, and the severity of dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS), and immune status was investigated in 245 Vietnamese infants with predominantly primary infections with dengue virus. Male and female infants were at equal risk of developing DHF/DSS. However, infants of low height and weight for age were under-represented among DHF/DSS cases compared with 533 healthy baby clinic infant controls. Acute illness phase blood levels of selected cytokines (interferon-γ and tumor necrosis factor-α) and serum levels of antibodies to dengue virus were elevated in the same range in male and female infants with DHF/DSS, as well as in infants with and without malnutrition.

INTRODUCTION

Dengue hemorrhagic fever (DHF)/dengue shock syndrome (DSS) is an important public health problem in southeast Asian and western Pacific countries. It is one of the leading causes of hospitalization and death among children in many tropical Asian countries.1–3 Patients with DHF may die of prolonged shock, massive bleeding (usually gastrointestinal bleeding), respiratory failure, and dengue encephalopathy.3 The vast majority of cases, nearly 95%, are among children less than 15 years of age; while infants comprise 5% or more of all DHF/DSS cases.4,5

A preponderance of DHF/DSS in Thai females ≥ 4 years old has been documented.6 Rates of infection with dengue (DEN) virus among boys and girls have been shown to be identical in countries endemic for DHF/DSS.6 The integrity and strength of the cell-mediated immune response should be correlated with the severity of DHF/DSS.7 It is known that malnutrition suppresses cellular immune responses. Anto and others8 and Thiasykorn and Nimmannitya9 have shown that even mild degrees of protein-calorie malnutrition serve to spare children from severe DHF/DSS. The immunopathogenesis of dengue virus infection, with an emphasis on the immune deviation, autoantibodies, and cytokine production has been proposed.10 In our previous report as part of study project of DHF in infants, we demonstrated the production of high levels of both proinflammatory cytokines (interferon-γ [IFN-γ] and tumor necrosis factor-α [TNF-α]) and anti-inflammatory cytokines (interleukin-10 [IL-10] and IL-6) in infants with DHF/DSS that was consistent with infection-enhancement due to acquired maternal antibody.11 Primary dengue virus infections in infants less than one year of age comprise a unique subgroup of patients with DHF/DSS.5 Here we further characterize attributes of the DHF syndrome observed in infants less than one year of age experiencing predominantly primary dengue virus infections.

MATERIALS AND METHODS

Patients. Two hundred seventy-two infants < 12 months old admitted to the Department of Dengue Hemorrhagic Fever of Children’s Hospital No. 1 (Ho Chi Minh City, Vietnam) from August 1997 to December 2002 with a clinical diagnosis of DHF according to the criteria of the World Health Organization (WHO) (1997) were enrolled in the study after parental or guardian consent was obtained.3 Dengue virus infections in the patients were confirmed by 1) a viral envelope and membrane (E/M)–specific capture IgM enzyme-linked immunosorbent assay (ELISA) and/or a nonstructural protein 1 (NS1) serotype-specific IgG ELISA at the Center for Disease Control in Taipei, Taiwan,12 or 2) a capture IgM ELISA at the Pasteur Institute in Ho Chi Minh City.13 Patients were under routine care of one or more of the authors. Basic demographic data and medical history were obtained and a detailed physical examination including measurement of weight and height was conducted by the authors, and subsequent progress was recorded on a standard data form. Five hundred thirty-three healthy infants who visited the outpatient department to receive vaccinations during the period 2001–2002 were examined as a control group for assessment of nutritional status. Dates of birth were recorded and measurements of weight and height were obtained. The mean age of control infants was seven months (range 2–11 months). Ethical approval of the study was obtained from the Scientific and Ethical Committee of Children’s Hospital No. 1 in Ho Chi Minh City.

Assessment of nutritional status of DHF patients and healthy control infants. Epi-Info 2000 1.1 software (Centers for Disease Control and Prevention, Atlanta, GA) was used to calculate the anthropometric indices such as weight-for-age (WA), height-for-age (HA), and weight-for-height (WH) of infants with DHF and healthy control infants. These nutritional measurements were converted to z-scores (weight-for-
age Z-score [WAZ]; height-for-age Z-score [HAZ]; and
weight-for-height Z-score [WHZ]), also referred to as SD
units, based on the National Center for Health Statistics/
World Health Organization reference. The Z-score cutoff
point to classify low anthropometric levels is 2 SD units below
the reference median for the three indices. The cutoff for very
low anthropometric levels is more than 3 SD units below the
median.

Sample collection. Paired blood samples were collected
from each patient in the study: one acute-phase and one con-
valvescent-phase. An acute-phase blood sample (2–3 mL) was
obtained at admission (days 3–7 after the onset of fever). A
convalescent-phase blood sample (2–3 mL) was obtained in
the convalescent phase (days 8–19 after the onset of fever).
Sera were separated as quickly as possible and stored at
−70°C until used.

Assessment of immune response of the patients. Immune
response of the patients was assessed by determining the ca-
pacity to produce cytokines, IgM antibody to dengue virus,
and/or NS1 serotype-specific IgG antibodies.

Capture IgM and IgG ELISAs. Capture IgM and IgG
ELISAs using diluted pooled virus antigens from culture su-
pernants of DEN 1-, DEN 2-, DEN 3-, DEN 4-, and Japa-
nese encephalitis (JE) virus-infected Vero cells as antigens
were performed to measure the IgM and IgG antibodies from
paired sera of 118 infants at the Center for Disease Control in
Taipei, Taiwan. The optical densities of culture supernants
of Vero cells with and without dengue virus infection were
designated the test absorbance and negative control value,
respectively, for each sample in the ELISA. Positivity was
determined by comparison with individual negative controls.
A positive sample had a ratio of test absorbance to negative
control ≥ 2.0, and a negative sample had a ratio < 2.0. For
serum samples with positive results in the capture IgM and
IgG ELISAs, a ratio of IgM to IgG ≥ 1.2 was defined as a
primary dengue virus infection, and a ratio < 1.2 was defined
as a secondary dengue virus infection.12 A capture IgM
ELISA using diluted dengue or JE virus antigens from in-
fected suckling mouse brain extracted by the sucrose-acetone
method and monoclonal antibody SLE 6B6C-1/HRP conjug-
ate was performed to measure the IgM antibodies in 154
infants following the protocol of the Centers for Disease Con-
trol and Prevention (Fort Collins, CO) at the Pasteur Institute
in Ho Chi Minh City.13

NS1 serotype-specific IgG ELISA. An NS1 serotype-
specific IgG ELISA using diluted NS1-containing culture su-
pernants of DEN 1-, DEN 2-, DEN 3-, DEN 4-, or JE
virus-infected Vero cells as antigens was performed to mea-
sure the NS1-specific IgG antibody from the sera of the pa-
tients. The enzyme activity was developed and the optical
density was determined.12

Cytokine assays. The plasma levels of six cytokines (IFN-γ,
TNF-α, IL-10, IL-6, IL-4, and IL-2) of the patients were mea-
sured simultaneously by the BD Human Th1/Th2 Cytokine
Cytometric Bead Array Kit-II (BD Biosciences, Pharmigen,
CA) in a 50-μL sample according to the instructions of the
manufacturer.11

Data and statistical analysis. Analysis of variance was used
to compare the statistical significance of differences in nor-
mally distributed data, while the Kruskal-Wallis test for two
groups was used if the variances in the samples differed.
Statistical analyses were performed with Epi-Info 2000 version
1.1 software. Differences with P values < 0.05 were consid-
ered significant.

RESULTS

Clinical findings of DHF/DSS patients in the study. Based
on the capture IgM ELISA, a dengue virus infection was
confirmed as the etiology for 245 of 272 infants hospitalized
with DHF. Among these infants, 182 were categorized as non-
shock DHF (grade I, 1 infant; grade II, 181 infants) and 63 as
DSS (grade III, 54 infants; grade IV, 9 infants). The mean age
of the patients was 6.8 months (range = 1–11 months). The
clinical and cytokine profiles of 107 of these patients have
been published, and serologic testing showed that almost all
(95.3%) of the patients had primary dengue virus infections.11
All patients had high continuous fever that lasted from 2 to 13
days, with a mean 5.2 days. Petaechiae on the skin and hepa-
tomegaly were observed in 244 (99.6%) and 238 (97.1%) pa-
tients, respectively. DSS was recorded in 63 (25.7%) patients
in whom there were 6 cases complicated with prolonged
shock. Gastrointestinal (GI) bleeding and respiratory failure
were noted in 14 (5.7%) and 13 (5.3%). Eighteen (7.3%)
patients had neurologic signs (dengue encephalopathy) mani-
fested by convulsions (12 cases), lethargy (7 cases), coma (6
cases), and focal neurologic sign (1 case).

Hemoconcentration, as shown by a ≥ 20% increase in the
hematocrit in reference to a convalescent value, was observed
in 224 (91.4%) patients. The remaining 21 (8.5%) patients
had a ≥ 10–19% increase in the hematocrit. Thrombocyto-
penia (platelet count ≤ 100 × 10^3/mm^3) was found in 230
(93.8%); the remaining 15 (6.1%) had platelet counts of
104–190 × 10^3/mm^3.

Association between sex, nutritional status, and the sever-
ity of DHF in infants. The male/female ratio for all DHF
infants was 138:107 (1.29:1); for DSS cases, it was 40:23 (1.73:
1). Among 533 healthy control infants, the male/female ratio
was 276:257 (1.07:1). The distribution of sex in infants with
DHF or DSS in the study is not different from that of healthy
control subjects (odds ratio [OR] = 1.20, 95% confidence
interval [CI] = 0.88–1.65, P = 0.2) (Table 1). There was no
sex bias associated with prolonged shock, GI bleeding, respi-
ratory failure, or encephalopathy (Table 2).

Of infants with DHF/DSS, only 17 (6.9%) were malnour-
ished (underweight) as assessed by WA on admission: 16 with
moderate malnutrition (–3 < WAZ < –2), and 1 with severe
malnutrition (WAZ ≤ –3). Height was measured in 218 of
245 infants. When assessed by HA and HAZ, 31 (14.2%) infants
had a ≥ 10–19% increase in the hematocrit. Thrombocyto-
penia (platelet count ≤ 100 × 10^3/mm^3) was found in 230
(93.8%); the remaining 15 (6.1%) had platelet counts of
104–190 × 10^3/mm^3.

SEX AND NUTRITIONAL STATUS IN INFANTS WITH DENGUE 371
TABLE 1
Association between sex, nutritional status, and the severity of dengue hemorrhagic fever (DHF) in infants*

<table>
<thead>
<tr>
<th>Findings</th>
<th>All patients (n = 245)</th>
<th>Nonshock DHF (n = 182)</th>
<th>DSS (n = 63)</th>
<th>Healthy controls (n = 533)</th>
<th>OR (95% CI), P†</th>
<th>OR (95% CI), P‡</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex, number (%)</strong></td>
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<td></td>
</tr>
<tr>
<td>Male</td>
<td>138</td>
<td>98 (71)</td>
<td>40 (28.9)</td>
<td>276</td>
<td>1.20 (0.88–1.65), 0.2</td>
<td>1.49 (0.79–2.81), 0.2‡</td>
</tr>
<tr>
<td>Female</td>
<td>107</td>
<td>84 (78.5)</td>
<td>23 (21.4)</td>
<td>257</td>
<td></td>
<td></td>
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<tr>
<td><strong>Nutritional status, number (%)</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Assessed by weight-for-age</td>
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<tr>
<td>Normal nutritional</td>
<td>228 (93.1)</td>
<td>169 (92.8)</td>
<td>59 (93.6)</td>
<td>471 (88.3)</td>
<td>0.55 (0.30–1), 0.03</td>
<td>0.88 (0.20–3.00), 1¶</td>
</tr>
<tr>
<td>Malnourished</td>
<td>17 (6.9)</td>
<td>13 (7.1)</td>
<td>4 (6.3)</td>
<td>62 (11.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Assessed by height-for-age</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal nutritional</td>
<td>201 (92.2)</td>
<td>160 (93.5)</td>
<td>41 (87.2)</td>
<td>412 (77.2)</td>
<td>0.29 (0.16–0.50), P &lt; 0.001</td>
<td>2.13 (0.61–6.71), 0.2‡</td>
</tr>
<tr>
<td>Malnourished</td>
<td>17 (7.7)</td>
<td>11 (6.4)</td>
<td>6 (12.7)</td>
<td>121 (22.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Assessed by weight-for-height</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Normal nutritional</td>
<td>187 (85.7)</td>
<td>149 (87.1)</td>
<td>38 (80.8)</td>
<td>527 (98.8)</td>
<td>3.20 (2.65–3.85), P &lt; 0.001</td>
<td>1.60 (0.63–4.04), 0.3§</td>
</tr>
<tr>
<td>Malnourished</td>
<td>31 (14.2)</td>
<td>22 (12.8)</td>
<td>9 (19.1)</td>
<td>6 (1.1)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* DSS = dengue shock syndrome; OR = odds ratio; CI = confidence interval.
† P values of comparison between all infants with DHF and healthy control infants by Yates’ corrected chi-square test.
‡ P values of comparison between nonshock DHF and DSS groups by ¥Yates’ corrected chi-square test or ¥two-tailed Fisher exact test.

were observed in the distribution of various parameters of undernutrition in the DSS and nonshock DHF group (Table 1).

Further analysis indicated that there was no association between malnutrition status as assessed by WA and severe complications (prolonged shock, GI bleeding, respiratory failure, and encephalopathy) in DHF infants (Table 2).

### TABLE 2
Association between sex and malnutrition as assessed by weight-for-age and severe complications in dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS) in infants*

<table>
<thead>
<tr>
<th>Severe complications</th>
<th>Male DHF/DSS infants (n = 138)</th>
<th>Female DHF/DSS infants (n = 107)</th>
<th>Malnourished DHF/DSS infants (n = 17)</th>
<th>Normal nutritional DHF/DSS infants (n = 228)</th>
<th>OR (95% CI), P†</th>
<th>OR (95% CI), P‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>GI bleeding</td>
<td>9</td>
<td>5</td>
<td>1</td>
<td>13</td>
<td>1.42 (0.41–5.57), 0.7§</td>
<td>1.03 (0.0–8.61), 1</td>
</tr>
<tr>
<td>Prolonged shock</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>5</td>
<td>0.55 (0.07–4.46), 0.6¶</td>
<td>3.49 (0.06–54.54), 0.3</td>
</tr>
<tr>
<td>Respiratory failure</td>
<td>6</td>
<td>7</td>
<td>1</td>
<td>12</td>
<td>0.65 (0.18–2.26), 0.6§</td>
<td>1.13 (0.0–9.47), 1</td>
</tr>
<tr>
<td>Hepatic failure</td>
<td>8</td>
<td>4</td>
<td>3</td>
<td>9</td>
<td>1.55 (0.48–5.01), 0.6§</td>
<td>3.47 (0.54–21.63), 0.1</td>
</tr>
<tr>
<td>Encephalopathy</td>
<td>9</td>
<td>9</td>
<td>2</td>
<td>16</td>
<td>0.76 (0.26–2.20), 0.7§</td>
<td>1.77 (0.0–9.35), 0.3</td>
</tr>
</tbody>
</table>

* GI = gastrointestinal; OR = odds ratio; CI = confidence interval.
† P values of comparison between male and female infants with DHF/DSS by ¥Yates’ corrected chi-square test and ¥two-tailed Fisher’s exact test.
‡ P values of comparison between infants with DHF/DSS with and without malnutrition by two-tailed Fisher’s exact test.
Comparison of serum levels of cytokines in acute-phase serum samples between male and female infants with dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS) and between infants with DHF/DSS with and without malnutrition as assessed by weight-for-age.

<table>
<thead>
<tr>
<th>Cytokine, mean ± SD pg/mL (range)</th>
<th>All patients (n = 62)</th>
<th>Male DHF/DSS infants (n = 35)</th>
<th>Female DHF/DSS infants (n = 27)</th>
<th>Malnourished infants (n = 27)</th>
<th>Normal nutritional infants (n = 36)</th>
<th>Controls (n = 6)</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN-γ</td>
<td>56.2 ± 115.4</td>
<td>(0–690.8)</td>
<td>46.7 ± 76.8</td>
<td>(0–351.3)</td>
<td>68.6 ± 152.5</td>
<td>(0–690.8)</td>
<td>237.7 ± 275.5</td>
</tr>
<tr>
<td>TNF-α</td>
<td>9.0 ± 13.2</td>
<td>(0–77.6)</td>
<td>9.5 ± 11.6</td>
<td>(0–77.6)</td>
<td>8.4 ± 15.4</td>
<td>(2.5–31.4)</td>
<td>19.2 ± 11.4</td>
</tr>
<tr>
<td>IL-10</td>
<td>73.8 ± 69.8</td>
<td>(0–210)</td>
<td>87.8 ± 83.4</td>
<td>(0–210)</td>
<td>55.6 ± 41.7</td>
<td>(2.1–123.2)</td>
<td>26.2 ± 20.2</td>
</tr>
<tr>
<td>IL-6</td>
<td>28.2 ± 41.7</td>
<td>(0–210)</td>
<td>33.4 ± 48.0</td>
<td>(0–210)</td>
<td>21.4 ± 31.3</td>
<td>(2.1–123.2)</td>
<td>26.2 ± 20.2</td>
</tr>
<tr>
<td>IL-4</td>
<td>2.0 ± 3.2</td>
<td>(0–17)</td>
<td>2.3 ± 3.6</td>
<td>(0–17)</td>
<td>1.5 ± 2.7</td>
<td>(0–10.3)</td>
<td>5.4 ± 3.7</td>
</tr>
<tr>
<td>IL-2</td>
<td>2.6 ± 4.7</td>
<td>(0–30)</td>
<td>3.5 ± 5.7</td>
<td>(0–30)</td>
<td>1.5 ± 2.4</td>
<td>(0–8.6)</td>
<td>4.1 ± 4.3</td>
</tr>
</tbody>
</table>

* IFN = interferon; TNF = tumor necrosis factor; IL = interleukin.
† P values of comparison between male and female infants with DHF/DSS and those with normal nutritional status by the Kruskal-Wallis test.
‡ P values of comparison between male and female infants with DHF/DSS with and without malnutrition by the Kruskal-Wallis test.
§ P values of comparison between male and female infants with DHF/DSS with and without malnutrition by the Mann-Whitney U test.

Comparison of levels of anti-dengue IgM, NS1 serotype-specific IgG antibodies between male and female infants with dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS) and between infants with DHF/DSS with and without malnutrition as assessed by weight-for-age.

<table>
<thead>
<tr>
<th>IgM ELISA/NS1 serotype-specific IgG ELISA (highest OD), mean ± SD (range)</th>
<th>All patients (n = 107)</th>
<th>Male DHF/DSS infants (n = 57)</th>
<th>Female DHF/DSS infants (n = 50)</th>
<th>Malnourished infants (n = 57)</th>
<th>Normal nutritional infants (n = 129)</th>
<th>Controls (n = 129)</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgM ELISA</td>
<td>At Center for Disease Control, Taipei, Taiwan</td>
<td>2.8 ± 1.1</td>
<td>2.9 ± 1.1</td>
<td>2.8 ± 1.0</td>
<td>2.8 ± 0.6</td>
<td>2.9 ± 1.1</td>
<td>0.6‡, 0.4‡</td>
</tr>
<tr>
<td></td>
<td>At Pasteur Institute, Ho Chi Minh City, Vietnam</td>
<td>1.8 ± 0.5</td>
<td>1.8 ± 0.5</td>
<td>1.7 ± 0.5</td>
<td>1.7 ± 0.8</td>
<td>1.8 ± 0.6</td>
<td>0.8‡, 0.6‡</td>
</tr>
<tr>
<td>NS1 serotype-specific IgG</td>
<td>ELISA</td>
<td>1.2 ± 0.6</td>
<td>1.1 ± 0.5</td>
<td>1.3 ± 0.6</td>
<td>1.5 ± 0.5</td>
<td>1.2 ± 0.6</td>
<td>0.3‡, 0.3‡</td>
</tr>
</tbody>
</table>

* NS1 = nonstructural protein 1; ELISA = enzyme-linked immunosorbent assay; OD = optical density. Levels of IgM antibody to dengue virus were measured in acute- and convalescent-phase serum samples, while NS1 serotype-specific IgG antibodies were measured in convalescent-phase serum samples.
† P values of comparison between male and female infants with DHF/DSS, with and without malnutrition by the Kruskal-Wallis test.
‡ P values of comparison between male and female infants with DHF/DSS with and without malnutrition by the Kruskal-Wallis test.
§ P values of comparison between male and female infants with DHF/DSS with and without malnutrition by the Mann-Whitney U test.
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Authors’ addresses: Nguyen Thanh Hung, Nguyen Trong Lan, Department of Dengue Hemorrhagic Fever, Children’s Hospital No.1, 341 Su Van Hanh Street, District 10, Ho Chi Minh City, Vietnam; Lam Thi My, Department of Pediatrics, University Hospital No.1, Ho Chi Minh City, Vietnam; and Phan Thanh Huy, Department of Microbiology and Immunology, College of Medicine, National Cheng Kung University, Tainan, Taiwan, Republic of China. Do Quang Ha and Vu Thi Que Huong, Arbovirus Laboratory, Pasteur Institute, Ho Chi Minh City, Vietnam; and Tuyen Thi Nguyen, Department of Medical Technology, College of Medicine, National Cheng Kung University, Tainan, Taiwan, Republic of China. Jyh-Hsiung Huang, Division of Research and Diagnosis, Center for Disease Control, Department of Health, Taipei, Taiwan, Republic of China. Scott B. Halstead, Uniformed Services University of the Health Sciences, Bethesda, MD 20815, E-mail: halsteads@erols.com.

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