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Hematuria is a marker for the severity of acute kidney injury but does not associate with thrombocytopenia in acute Puumala hantavirus infection

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Abstract

Backgound: Puumala hantavirus (PUUV) causes hemorrhagic fever with renal syndrome characterized by thrombocytopenia, capillary leakage and acute kidney injury (AKI) with proteinuria and hematuria. Although the typical histologic lesion is acute tubulointerstitial nephritis, the amount of glomerular proteinuria predicts the severity of upcoming AKI. Here we studied the associations of hematuria and proteinuria with the severity of emerging AKI, thrombocytopenia, and markers of coagulation and fibrinolysis in PUUV infection.

Methods: We examined 205 consecutive patients treated for serologically confirmed acute PUUV infection at Tampere University Hospital during 1997-2014. The patients were divided into three groups according to the combined positive result in urine hemoglobin and albumin dipstick tests: 0-2+ (n=58), 3-4+ (n=100), and 5-6+ (n=47).

Results: The medians of maximum creatinine concentrations in the three groups were: 0-2+ 100 μmol/L (range 52-1499), 3-4+ 204 μmol/L (range 65-1071), and 5-6+ 361 μmol/l (range 51-1285) ($p<0.001$). The number of blood platelets ($p=0.069$), and the levels of fibrinogen, prothrombin fragments F1+2, and d-dimer ($p=0.602$, $p=0.113$, $p=0.289$, respectively) were not significantly different between the groups. When the amount of hematuria in the dipstick test was examined separately, no association with thrombocytopenia was detected ($p=0.307$ between groups 0, 1+, and 2-3+).

Conclusions: Combined positive result of hematuria and proteinuria in the dipstick test at hospital admission predicted the severity of upcoming AKI in acute PUUV infection. As hematuria was not associated with the severity of thrombocytopenia, it did not indicate increased bleeding tendency, but was rather a marker of acute kidney injury.

Introduction

Hantaviruses infect many species of rodents, shrews, moles, and bats and, in humans, cause hemorrhagic fever with renal syndrome (HFRS) in Europe and Asia and hantavirus cardiopulmonary syndrome (HCPS) in the Americas [1]. Puumala virus (PUUV), carried by the bank vole, causes a HFRS called nephropathia epidemica (NE) [1]. Most of the European HFRS cases are caused by PUUV and the majority of all NE cases are reported in Finland, where thousands of serological diagnoses are made annually [2].

The typical features in all HFRS cases are fever, increased vascular permeability, renal involvement, and thrombocytopenia [3, 4]. Renal involvement in PUUV infection causes temporarily decreased glomerular filtration, transient, often massive, proteinuria, hematuria, and oliguria, which is followed by polyuria and spontaneous recovery [3-5]. Smokers have a more severe acute kidney injury (AKI) than non-smokers [6, 7]. However, the outcome of AKI in PUUV infection is favorable [8].

Despite often substantial thrombocytopenia, serious hemorrhages are rare, while mild bleeding manifestations, such as conjunctival bleeding, epistaxis, or petecchiae, occur in about one-third of the patients [3]. The mortality of PUUV infection is low, ranging from less than 0.1% in Finland to 0.4% in Sweden [9, 10]. However, the disease often leads to hospitalization and intensive care unit treatment, including renal replacement therapy, may be needed [3].

The characteristic histopathologic renal finding in PUUV infection is acute tubulointerstitial nephritis, and the infiltrating cells include lymphocytes, plasma cells, monocytes, macrophages, and polymorphonuclear cells [11, 12]. Glomerular changes are mild and do not correlate with the amount of proteinuria [11, 12]. Medullary hemorrhages have been found in 20% of renal biopsy samples and almost all medulla-containing biopsy specimens have revealed hemorrhages [11-13].

Microscopic hematuria is present in a vast majority (58-94%) of patients infected by PUUV [5, 11, 12, 14]. Recently, albuminuria was found to predict the severity of upcoming AKI [15]. However, the significance of hematuria in PUUV infection has not been established. In this study, we examined the associations of hematuria, and combined hematuria and albuminuria, with emerging AKI, thrombocytopenia, and markers of coagulation and fibrinolysis in acute PUUV hantavirus induced HFRS.

Materials and Methods

Subjects

The study cohort consisted of 217 consecutive patients with acute PUUV infection, who also participated in our previous studies [6, 8, 14-16]. The patients were treated at the Tampere University Hospital, Finland, during 1997-2014. All patients were examined during the acute phase of the disease, and the diagnosis of acute PUUV infection was serologically confirmed in all of them [17-19]. A detailed past and current medical history was obtained and careful physical examination performed. The median patient age was 41 (range 15-77) years, 12 patients (7%) were older than 65 years, and 138 patients (67%) were males. Patients younger than 15 years of age were excluded from the study as well as patients with a missing dipstick test. Altogether 205 patients were included in the analyses.

Fifty-six patients (27%) had one or more diseases diagnosed before PUUV infection. The diseases have been described in detail earlier [15]. None of the patients had any known chronic kidney disease prior to PUUV infection. One patient had suffered from renal tuberculosis and another patient had polycystic kidney disease, but they did not have chronic impairment of kidney function. All patients provided a written informed consent and the study was approved by the Ethics Committee of Tampere University Hospital.

Laboratory determinations

In all 205 patients, urine dipstick test was performed at hospital admission, and daily plasma creatinine and platelet values during the hospital stay were determined. The amount of hematuria detected by dipstick test was graded into three categories: 0 (28 patients), 1+ (94 patients), and 2-3+ (83 patients). Urine sediment was also assessed in 189 patients, either by microscopy or by flow cytometry and coefficient factor 1 erythrocyte/high power field = 5.8 x 10^6/L was used to standardize the results.

Recent PUUV infection was confirmed from a single serum sample by detecting the typical granular staining pattern in immunofluorescence assay [17], and/or low avidity of IgG antibodies to PUUV [19], and/or by detecting PUUV IgM antibodies by an 'in-house' enzyme-linked immunosorbent assay (ELISA) based on a recombinant antigen [18].

Plasma creatinine was determined until 1999 by Vitros (Johnson & Johnson, Rochester, N.Y., USA) and thereafter by Cobas Integra (F. Hoffman- La Roche Ltd., Basel, Switzerland). Urine dipstick analysis was made by automated tests based on refractometry: From 1997 using Miditron M (Roche), from 2004 Urisys 2400 or 1900 (Roche), and from 2009 until 2014 Siemens Clinitec Atlas or Advantus. Urine for the dipstick test was sampled on admission already at the emergency room.

Blood cell count was determined by hematological cell counters (Bayer Diagnostics, Elkhart, IN, USA). In 43 patients, plasma fibrinogen levels were assessed from citrate-anticoagulated samples after centrifugation at 2000 g for 10 min at room temperature using a viscosity-based detection system (Diagnostica Stago; reference range 2.0–4.0 g/l). D-dimer (Tina-quant D-Dimer immunoturbidimetric assay, Roche Diagnostics, Mannheim, Germany) and prothrombin fragments

(F1+2, a monoclonal enzyme immunoassay Enzygnost F1+2, Siemens Healthcare Diagnostics) were also assessed in the same 43 patients.

In 70 patients, urine collection was started on the first evening of hospital care and continued for 3 days. The nightly collection period was from the time of the last voiding at bedtime until the last voiding on rising. The 24-hour collection commenced immediately thereafter. The 24-hour urinary protein excretion was measured by the pyrogallol red molybdate method (Olli C.; Kone Instruments, Helsinki, Finland) until 1998, then by using Cobas Integra (Roche diagnostics) until 2008, and thereafter using Cobas 8000 analyzer (Roche diagnostics). Timed overnight urinary excretion of albumin and a1-microglobubulin was measured using nephelometry (Behring Nephelometer II Analyzer, Behringwerke AG, Marburg, Germany).

Other analytical procedures were performed using routine automated chemistry analyzers. The highest and lowest values of the variables measured during hospitalization for each patient were designated as the maximum and minimum values, respectively. All laboratory determinations were performed by the laboratory Centre of the Pirkanmaa Hospital District (later named Fimlab Laboratories), Tampere, Finland.

Statistical analyses

Medians and ranges were given for skewed continuous variables and numbers and percentages for categorical variables. Spearman's rank correlations were calculated. Categorical data were analyzed using the x^2 test or the Fisher's exact test, and groups were compared using the Mann-Whitney *U*-test or the Kruskal-Wallis test, as appropriate. All tests were two-sided, and the analyses were performed using SPSS (version 20) statistical software (IBM, Chicago, IL).

Results

The clinical characteristics and laboratory findings of the patients have been described earlier [15]. The patients were admitted to the hospital median 4 (range 1-15) days after the onset of fever. Median duration of the hospital stay was 6 (range 2-25) days. Six patients (3%) were in clinical shock at admission, while 9 patients (4%) needed renal replacement therapy during hospitalization. All patients recovered.

Altogether 177 patients (86%) had hematuria detected by dipstick test (1-3+) at admission. In 94 patients (46%) hematuria was mild, categorized as 1+, while 83 patients (40%) had grade 2-3+ hematuria. In 189 patients (92%) also urine sediment was examined. The amount of erythrocytes in the sediment was elevated (>2/high power field) in 122 (65%) patients.

The degree of hematuria assessed by dipstick associated with the severity of AKI. Figure 1 shows the association of the degree of dipstick-verified hematuria with maximum plasma creatinine level. Also, the amount of erythrocytes in the sediment correlated slightly with the severity of AKI ($r=0.168$ for correlation with maximum plasma creatinine, $p=0.021$). The associations of dipstick-verified hematuria with various clinical and laboratory variables are shown in Table I. Patients with more pronounced hematuria had greater change in weight during hospitalization (reflects fluid retention during the oliguric phase), lower minimum urinary output, and higher maximum plasma creatinine and urea concentrations. Patients with higher degree of hematuria also had lower minimum albumin and sodium concentrations, and lower minimum hematocrit. The inflammatory variables C-reactive protein (CRP) and leukocyte count did not associate with the amount of dipstick-verified hematuria. Higher dipstick-verified hematuria associated with greater urinary excretions of 24-hour protein and overnight albumin, but not with that of overnight urinary α1-microglobulin (i.e. tubular proteinuria) (Table I).

There was no association of dipstick-verified hematuria with thrombocytopenia. The coagulation and fibrinolysis markers fibrinogen, prothrombin fragments F1+2, and d-dimer had no association with the amount of hematuria assessed by dipstick (Table I) or erythrocytes in the urine sediment (data not shown). Minimum blood platelet count did not associate with the amount of erythrocytes in the sediment ($r=-0.124$, $p=0.088$). The amount of hematuria measured by dipstick test associated with the amount of erythrocytes in urine sediment (Table I).

The amount of dipstick-verified hematuria (grading 0-3+) was associated with the amount of dipstick-verified albuminuria (grading 0-3+) ($p<0.001$). Out of the patients, 57% with grade 3+ hematuria had also grade 3+ albuminuria, whereas none of the patients without dipstick-verified hematuria (grade 0) had grade 3+ albuminuria. The amount of dipstick-verified hematuria did not associate with the amount of dipstick-verified leukocyturia ($p=0.749$).

To further examine the associations of dipstick-verified hematuria with the severity of AKI, we calculated the influence of combined positive results in the dipstick hematuria and albuminuria tests (range from 0 to 3+ in each). With every positive semiquantitative step further (combined range outcome from 0 to 6+) in these tests, the plasma creatinine level rose: 0 creatinine 94 µmol/L (range 58-874), 1+ creatinine 101 µmol/L (range 58-1499), 2+ creatinine 102 µmol/L (range 52-541), 3+ creatinine 138 µmol/L (range 65-841), 4+ creatinine 256 µmol/L (range 69-1071), 5+ creatinine 361 µmol/L (range 51-1285), 6+ creatinine 426 µmol/L (range 160-1156) ($p<0.001$). Thereafter, we divided the patients into three groups according to the combined results of these two dipstick tests: 0-2+ (58 patients), 3-4+ (100 patients), and 5-6+ (47 patients) (Table II). Higher number of positive results in these two tests was associated with more severe upcoming AKI, and the patients stayed longer at the hospital. Overnight urinary albumin excretion also associated with higher number of positive results in the dipstick tests. Blood platelet level or the coagulation and fibrinolysis markers did not associate with the combined number of positive dipstick test results.

Discussion

To our knowledge this is the first study in which hematuria was systematically examined with respect to the severity of the emerging AKI and glomerular and tubular proteinuria during hantavirus infection, and the possible relation to laboratory findings reflecting hemostasis was also addressed. The present study showed that hematuria in acute PUUV hantavirus infection was associated with the severity of AKI, but not with thrombocytopenia or markers of coagulation and fibrinolysis. Furthermore, this study pointed out that when evaluating the severity of the developing AKI, both dipstick-verified hematuria and albuminuria should be considered.

Hematuria was detected by dipstick test in 86% of the 205 patients in the present study. This is well in line with previous findings, where microscopic hematuria was found in the majority, 58-94%, and gross hematuria in 3% of patients with PUUV infection [3, 5, 11, 14]. Altogether, hematuria is far more common in NE than in other types of acute tubulointerstitial nephritis, in which only about one-third of the patients present with hematuria [20].

We found that the degree of hematuria detected by dipstick test was associated with the severity of the upcoming AKI. The degree of dipstick-verified hematuria also associated with albuminuria measured by dipstick, 24-hour urine protein excretion, and overnight albumin excretion. Dipstick-verified hematuria did not associate with overnight α1-microglobulin excretion. Recently, we found that glomerular proteinuria, but not tubular proteinuria (α1-microglobulin), predicted the severity of AKI in NE [15]. More pronounced dipstick-verified hematuria was related to greater change in body weight, as well as lower minimum hematocrit, albumin, and sodium levels. All of these variables probably reflect fluid retention during the oliguric phase of PUUV infection, and they can be considered as consequences of more severe AKI.

Previously, a slight but significant correlation between the degree of microscopic hematuria and maximum creatinine level in PUUV infection was reported, in agreement with the present results [12]. Hematuria has also been associated with the development of polyuria, a typical sign of renal involvement in PUUV infection [21]. Furthermore, in HFRS caused by Hantaan virus, the presence of microscopic hematuria associates with the risk for developing oliguric AKI [22]. On the other hand, in a study aimed at identifying patients who were at lower risk of developing severe AKI, hematuria was not found to be a risk factor for severe AKI when compared with mild to moderate AKI [23]. However, the research approach was different from our study and the number of patients was lower, which may explain the divergent results.

In the present study, the amount of hematuria verified by dipstick test was not associated with inflammatory markers, i.e. blood leukocyte and CRP levels. Moreover, hematuria did not associate with the levels of blood platelets or markers of coagulation and fibrinolysis. In concordance with the present results, a previous German study did not find a difference in the presence of hematuria between patients with severe and non-severe thrombocytopenia [24]. PUUV infection is a HFRS with mild bleeding tendency. However, some bleeding manifestations, e.g. conjunctival bleeding, epistaxis, or petecchiae, occur in about one-third of the patients [3]. There are also case reports of serious bleedings, such as hypophyseal hemorrhages [25], while medullary hemorrhages are common findings in renal biopsy specimens [12]. In theory, hematuria could well be a sign of bleeding tendency in this HFRS, but our findings suggest that this is not the case. In our previous study, thrombocytopenia was not found to associate with AKI [16]. This is in line with the findings of the present study, where hematuria associated with AKI, but not with thrombocytopenia.

Previously, the amount of hematuria has not been found to be related to the extent of renal histologic findings [12]. It cannot be, however, excluded that medullary hemorrhages contribute to the occurrence of hematuria during PUUV infection. When evaluating previous results regarding the relationship between the amount of hematuria and findings in renal biopsies, it must be taken into

account that kidney biopsies have usually been performed after the acute-phase thrombocytopenia has resolved to avoid biopsy-related bleeding complications. Urine samples, on the contrary, have typically been collected already on admission to the hospital. Thus, the timing of the biopsies may have influenced the results.

The amount of erythrocytes in urine sediment was associated with dipstick-verified hematuria. Urine sediment erythrocyte count also correlated with the severity of AKI, but not with platelet count or the levels of the markers of coagulation and fibrinolysis. Thus, hematuria measured using this method provided corresponding results to the semi-quantitative dipstick hematuria test.

The significance of combined positive results for dipstick-verified hematuria and albuminuria was also analyzed. Higher number of positive results in these two tests was associated with higher plasma concentration of maximum creatinine. The minimum hematocrit, albumin, and sodium concentrations were, in turn, lower with higher number of positive dipstick tests, probably reflecting fluid retention and the severity of AKI. When evaluating the significance of hematuria and albuminuria together, the predictive value for the upcoming AKI was even better than that of hematuria alone. Blood platelet count or the levels of coagulation and fibrinolysis markers did not associate with the number of positive dipstick test results, and also failed to predict the severity of AKI in this patient population.

In conclusion, the number of combined positive results in urine hemoglobin and albumin dipstick tests at hospital admission predicted the severity of the upcoming AKI during acute PUUV hantavirus infection. Hematuria alone was associated with the severity of AKI, but not with the severity of the emerging thrombocytopenia. This suggests that hematuria did not indicate higher bleeding tendency, but was rather a marker of kidney injury during acute hantavirus infection.

Table I Clinical and laboratory findings in 205 patients with Puumala hantavirus infection divided into three groups according to urine hemoglobin dipstick category.

	U-Eryt 0 **n=28**	**U-Eryt 1+** **n=94**	**U-Eryt 2-3+** **n=83**	**p-value**
Hospital stay (days)	5.5 (3-22)	6.0 (3-25)	6.0 (2-16)	0.181
Change in body weight (kg)	1.5 (0-9.4)	2.1 (0-10.7)	2.8 (0-12.0)	0.015
Urinary output min (ml/day)	1640 (0-5720)	1540 (100-4920)	1060 (50-7000)	0.034
Laboratory findings in plasma and blood				
P-Creatinine max (µmol/L)	99 (58-874)	175 (52-1499)	265 (51-1285)	0.001
P-Urea max (mmol/L) (n=110)	7.7 (2.7-20.3)	17.7 (2.1-52.4)	22.1 (2.7-52.8)	0.002
P-Alb min (g/L)	32 (11-37)	29 (20-39)	26 (18-39)	0.002
P-Sodium min (mmol/L)	135 (126-139)	132 (114-142)	131 (109-141)	0.005
Hematocrit min	0.38 (0.25-0.43)	0.36 (0.25-0.43)	0.35 (0.22-0.46)	0.022
Leukocytes max ($x10^9$/L)	9.8 (4.2-38.6)	10.7 (5.4-31.2)	10.4 (4.4-45.0)	0.443
P-C-reactive protein max (mg/L)	74 (16-236)	75 (20-244)	84 (16-269)	0.436
Laboratory findings in urine				
dU-Protein max (g/day) (n=70)	0.7 (0.3-3.7)	2.6 (0.3-10.0)	1.8 (0.1-17.8)	0.019
cU-Albumin max (µg/min) (n=70)	97 (14-738)	987 (14-7026)	738 (4-6246)	0.005
cU-α1-microglobulin max (µg/min) (n=70)	42 (20-89)	21 (7-130)	27 (7-209)	0.280
U-Erythrocytes (per high power field)	2 (1-132)	3 (0-41)	8 (0-401)	<0.001
Laboratory findings reflecting hemostasis				
Platelets min ($x10^9$/L)	63 (19-172)	67 (13-249)	56 (3-198)	0.307
P-Fibrinogen (g/L) (n=43)	4.2 (2.0-9.6)	4.2 (2.2-6.7)	4.5 (1.5-5.7)	0.863
P-Prothrombin fragments F1+2 (pmol/l) (n=43)	542 (329-1160	583 (149-1487)	711 (284-1875)	0.811
P-D-dimer (mg/L) (n=43)	1.4 (0.6-29.6)	3.4 (0.3-13.5)	3.3 (0.8-34.0)	0.405

Values are medians (ranges). P=plasma, U=urine, dU=daily urine, cU=overnight urine collection, min=minimum, max=maximum.

Table II The clinical and laboratory findings in 205 patients with Puumala hantavirus infection divided into three groups according to combined positive result in urine hemoglobin and albumin dipstick tests.

	0-2+ n=58	**3-4+ n=100**	**5-6+ n=47**	**p-value**
Hospital stay (days)	5.5 (3-25)	6.0 (3-12)	7.0 (2-15)	0.022
Change in body weight (kg)	1.6 (0-10.7)	2.2 (0-10.8)	3.8 (0-12.0)	<0.001
Urinary output min (ml/day)	1900 (0-5720)	1440 (100-4900)	780 (50-7000)	<0.001
Laboratory findings in plasma and blood				
P-Creatinine max (µmol/L)	100 (52-1499)	204 (65-1071)	361 (51-1285)	<0.001
P-Urea max (mmol/L) (n=110)	9.4 (2.1-52.4	17.7 (3.9-39.7)	24.6 (2.7-52.8)	0.001
P-Sodium min (mmol/L)	135 (114-141)	132 (109-142)	129 (113-141)	<0.001
P-Alb min (g/L)	30 (11-39)	28 (22-37)	25 (18-36)	0.001
Hematocrit min	0.37 (0.22-0.43)	0.36 (0.25-0.44)	0.35 (0.25-0.46)	0.109
Leukocytes max ($x10^9$/L)	9.6 (4.2-38.6)	10.4 (5.7-31.2)	13.4 (4.4-45.0)	0.001
P-C-reactive protein max (mg/L)	78 (16-236)	81 (16-269)	80 (22-214)	0.624
Laboratory findings in urine				
dU-Protein max (g/day) (n=70)	1.2 (0.1-9.5)	1.9 (0.2-17.8)	2.0 (0.2-7.6)	0.318
cU-Albumin max (µg/min) (n=70)	97 (4-4617)	902 (12-7026)	1018 (55-4358)	0.011
cU-α1-microglobulin max (µg/min) (n=70)	20 (10-89)	25 (7-209)	27 (7-112)	0.694
U-Erythrocytes (per high power field)	2 (0-132)	4 (0-41)	14 (0-401)	<0.001
Laboratory findings reflecting hemostasis				
Platelets min ($x10^9$/L)	63 (3-249)	66 (15-198)	52 (5-187)	0.069
P-Fibrinogen (g/L) (n=43)	4.2 (2.0-9.6)	4.1 (2.8-6.7)	4.8 (1.5-5.7)	0.602
P-Prothrombin fragments F1+2 (pmol/l) (n=43)	534 (295-1160)	614 (149-1487)	769 (429-1875)	0.289
P-D-dimer (mg/L) (n=43)	1.4 (0.6-29.6)	3.6 (0.3-8.7)	3.8 (1.1-34.0)	0.113

Values are medians (ranges). P=plasma, U=urine, dU=daily urine, cU=overnight urine collection, min=minimum, max=maximum.

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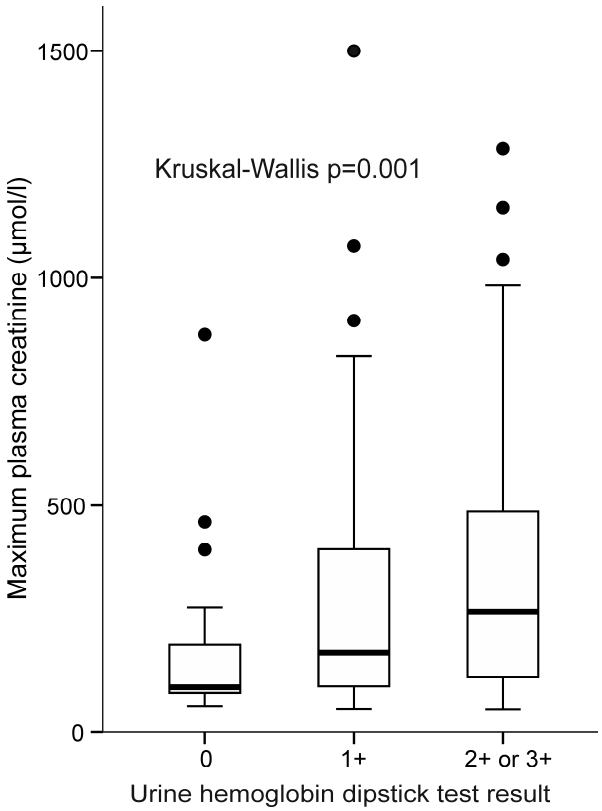
Figure 1. Maximum plasma creatinine level during hospital care in relation to urine hemoglobin dipstick test result at the entry to the hospital.



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Disclosure of interest

The authors report no conflicts of interest.

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