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A Study on Thrombokinetics in Korean Hemorrhagic Fever*

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= Abstract = To elucidate the cause of thrombocytopenia in Korean hemorrhagic fever, platelet counts, ^{111}In-labeled allogeneic platelet survival and bone marrow changes were assessed in the early stage in thirteen male patients with follow-up evaluation six to eight days later. ^{111}In-labeled allogeneic platelet survival was decreased to 111.9 ± 21.9 hours in the early stage when the mean platelet count was $54,000 \pm 28,000/mm^3$, compared with 147.8 ± 18.2 hours in normal controls ($p < 0.01$). Significant increase of the megakaryocyte number was observed in the bone marrow of the early stage patients while the bone marrow cellularity showed no significant changes. The megakaryocyte size revealed no significant changes. Significant correlations were found among the platelet survival, platelet count, megakaryocyte number and/or megakaryocyte size in the early stage of the disease. It was concluded that thrombocytopenia in the early stage of Korean hemorrhagic fever results from increased consumption, but not from impaired production, of the platelets.

Key Words: ***Korean hemorrhagic fever, Platelet survival, Megakaryocyte***

INTRODUCTION

Korean hemorrhagic fever is an acute viral disease characterized by fever, hemorrhagic manifestations and renal failure occuring from the Pacific Ocean to the Baltic sea under various synonyms and toponyms. Recent serologic investigations demonstrated the identity of the similar conditions described from Korea, the soviet Union (Lee et al. 1978), Japan (Lee et al. 1979) and China (Lee et al. 1980).

Disseminated intravascular coagulation has been suggested to be responsible to some degree for the bleeding tendency which comprises one of the most conspicuous findings in the early stage of the disease (Dennis and Conrad 1968; Bunin and Abdurashitov 1976; Lee 1976; Sirotin et al. 1977; Lee et al. 1981). Concerning the cause of thrombocytopenia, however, different views have been proposed. Some insisted on accelerated destruction of platelets (Paik 1974; Moon et al. 1975; Lee et al. 1981) while others suggested the possibility of thrombopoietic failure in bone marrow as well (Park et al. 1973; Chi et al. 1976; Hong et al. 1976; Lee and Lee 1979).

We think this controversy results from the following reasons. First, previous studies were not performed at the same stage while the changes in coagulation system is so rapid in the early stage of this disease (Lee et al. 1981). Second, most of the previous marrow observations were not made on histologic sections but on aspirate smears. Accurate assessment of the megakaryocyte production requires bone marrow biopsies (Wickramasinghe 1975). Finally, to date, there has not been a suitable label for thrombokinetic studies. Profound thrombocytopenia and bleeding tendency have made these procedures even more difficult to be performed.

The purpose of this study was to elucidate the cause of thrombocytopenia in the early stage of Korean hemorrhagic fever through the sequential thrombokinetic studies and histological evaluation of the bone marrow sections.

MATERIALS AND METHODS

Materials:

Thirteen male patients with Korean hemorrhagic fever from the 1983 epidemic in Korea were in-

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cluded in this study. They were 22 to 24 years old. Only those in febrile/hypotensive or early oliguric phase of the illness were selected. Day of illness counted from the day of symptom onset ranged from 5 to 7. The diagnosis was established by clinical features with serologic confirmation.

Control platelet survival determinations were made in eight young male volunteers, 22 to 33 years of age. None of the patients and control subjects had a history of transfusion with any blood component prior to the tests.

Control values for the number and size of the megakaryocytes in bone marrow were determined in six patients, 21 to 28 years of age showing normal hemogram and no evidnce of bone marrow involvement with the underlying diseases (four non-Hodgkin's lymphomas of stage I or II, one Hodgkin's disease of stage I and one non-specific lymphadenitis).

Methods:

Measurement of the paltelet counts and platelet survival, and bone marrow examinations were made in febrile/hypotensive or early oliguric phase (early stage) with follow-up assessment six to eight days later (later stage).

Platelet counts were measured with an electronic counter (TOA Platelet Counter PL-100). Platelet survival was determined according to a method modified from that recommended by the Panel on Diagnostic Application of Radioisotopes in Hematology of the International Committee for Standardization in Hematology (1977). Allogeneic platelets were labeled with ^{111}In-oxine utilizing a method similar to that described by Thakur et al. (1977). The procedure was performed in closed sterile tubes with plastic caps at room temperature and with pyrogen-free and sterile reagents. Cells or supernatant were removed with syringes having sterile needles.

Twenty ml of platelet-rich plasma was collected into a plastic tube via Haemonetics 30 S Blood Processor from a normal HB_sAg- and VDRL-negative and Rh-compatible group O donor. The tube was then centrifuged at 300 g for 15 min. Appropriate volume of the supernatant plasma containing about 7.5×10^9 platelets was transferred into another tube. After 1/20 volume of ACD (pH 6.5) was added, the platelets were sedimented into the button by centrifugation at 1500 g for 15 min. The supernatant platelet-poor plasma was saved in another tube. Five ml of ACD-saline (1:7 V/V) was added into the button and the platelets were resuspended by repeated gentle inversion of the tube. The platelets were sedimented again into the button by centrifugation at 1500 g for 15 min and the supernatant solution was discarded. The platelets were resuspended in 5 ml of ACD-saline by repeated gentle inversion. Three hundred μCi of ^{111}In-oxine (Amersham, Buckinghamshire, England) was added into the platelet suspension and the mixture was incubated at room temperature for 30 min. The labeled platelet suspension was centrifuged at 1500 g for 15 min and the supernatant was discarded. The platelet button was resuspended in 5 ml of platelet-poor plasma obtained from the previous step by repeated gentle inversion. The suspension was centrifuged at 1500 g for 15 min and the supernatant was discarded. the platelet button was resuspended in 5 ml of ACD-saline by repeated gentle inversion. The prepared labeled platelet suspension containing 108 to 220 μCi was injected intravenously and the time noted.

Blood samples were taken in EDTA bottles at one hour after injection and thereafter daily for six days. Daily samples were taken at the same time of day. Radioactivity in the whole blood of each sample was counted at the same time after collection in Packard 5210 gamma counter. The weighed mean platelet survival was calculated by the method recommended by the Panel on diagnostic Application of radioisotopes in Hematology of the International Committee for Standardization in Hematology(1977) using a personal computer (Sambo Trigem 20).

Bone marrow samples were obtained at the same day when the radiolabeled platelets were injected. Bone marrow biopsies were performed from the posterior iliac crests with a Jamshidi biopsy needle. Biopsy specimens were fixed in formalin and prepared according to the usual proceudres to be blocked in paraffin. Sections were cut at 4 μm thickness, mounted on cover slips, and stained with Periodic Acid-Schiff (PAS) by the routine method.

The ratio of the megakaryocytes to all nucleated cells in bone marrow was determined by counting at least 7,000 nucleated cells and the corresponding megakaryocytes in duplicate on random-section photomicrographic enlargement of the bone marrow sections. The megakaryocyte number was expressed as the number per 10^6 nucleated cells. The corrected megakaryocyte number was defined as the product of marrow cellularity (%) and mega-

Table 1. Clinical data of patients with Korean hemorrhagic fever

Case No.	Day of Illnesss		Age/Sex	Hemoglobin (gm%)		Hematocrit (%)		WBC (/mm^3)		BUN (mg%)		Creatinine (mg%)	
	Early	Later	(yr)	Early	Later	Early	Later	Early	Later	Early	Later	Early	Later
1	5	13	23/M	18.6	12.7	55	38	24,500	14,200	40.2	144.3	2.8	10.3
2	5	13	22/M	20.0	13.0	60	39	27,600	15,100	56.2	—	3.8	—
3	5	13	23/M	15.5	8.0	46	24	11,000	12,100	54.9	96.4	1.7	10.5
4	5	13	22/M	14.8	13.4	44	40	30.200	6.200	104.0	103.0	8.0	12.6
5	5	13	23/M	16.0	12.8	48	38	20,900	7,700	86.4	29.3	5.0	1.0
6	7	14	23/M	14.1	11.4	42	43	28,700	10,700	78.0	—	5.6	—
7	5	11	24/M	11.8	—	35	—	13,100	—	100.6	—	9.9	—
8	5	12	23/M	18.4	13.3	55	40	30,200	14,000	65.6	245.8	5.7	11.3
9	4	12	23/M	17.4	13.0	52	39	20,300	9.500	38.2	—	2.0	—
10	6	13	23/M	17.6	13.3	53	40	19.200	12,700	64.3	23.9	4.3	2.3
11	5	12	24/M	14.7	—	44	—	20,000	—	80.8	187.6	7.4	19.7
12	5	12	24/M	17.5	13.1	53	39	16,000	6,800	25.7	9.0	1.6	0.9
13	—	—	23/M	—	—	—	—	—	—	—	—	—	—

karyocyte number per 10^6 nucleated marrow cells. Bone marrow cellularity was assessed by six investigators working independently and blind.

The megakaryocyte size (S) was calculated from the longest and shortest diameters (r and r′ respectively) of more than 30 megakaryocytes containing a portion of the nucleus using the following formula;

$$S=3.14\times r/2\times r'/2$$

Statistical analysis:

The Student's t-test was used on paired data for intragroup (interstage) comparison and unpaired data for intergroup comparison. A linear regression analysis was performed between the various parameters tested in this study. Values are expressed as means±the standard deviation(SD) of the mean (Hill 1977).

RESULTS

In the early stage of Korean hemorrhagic fever, most patients showed increases in hematocrit and hemoglobin concentrations reflecting a loss of plasma through damaged capillaries. Leukocytosis with an increased number of myeloid elements was found in all patients. Moderate to severe increases in blood urea nitrogen and creatinine concentrations were observed in the early stage of the disease indicating that uremia had already developed at this stage. Six to eight days later when the follow-up assesement of platelet kinetic studies was made, most of the abnormal findings returned to normal whereas some patients developed progressive uremia (Table 1).

Table 2. Platelet counts in patients with Korean hemorrhagic fever

Case No.	Platelet Count($\times 10^4$/mm^3)	
	Early	Later
1	5.9	20.8
2	3.2	14.1
3	3.3	28.2
4	1.5	27.7
5	6.3	24.2
6	5.1	15.9
7	5.3	26.7
8	7.7	38.6
9	5.3	11.7
10	12.2	31.8
11	3.5	30.3
Mean±SD	5.4±2.8	24.5±8.2

The mean platelet count with standard deviation in the early stage was 54,000±28,000/mm^3. In the later stage, however, the count increased to 245,000±82,000/mm^3 ($p<0.01$) (Table 2).

^{111}In-labeled allogeneic platelet survival in the early stage was decreased to 111.9±21.9 hours compared with 147.8±18.2 hours in eight normal controls ($p<0.01$). The mean platelet survival was recovered to 157.2±18.8 hours in the later stage ($p<0.01$). The platelet survival in the later stage, however, was not statistically different from the value in normal controls ($p>0.20$) (Table 3 and 4, Fig. 1).

The cellularity of bone marrow was 52±17%

Table 3. Platelet survival in normal subjects

Case No.	Age/Sex (yr)	Blood Group		Survival (hr)
		Rh	ABO	
1	23/M	+	B	180.4
2	24/M	+	O	165.1
3	33/M	+	O	143.2
4	26/M	+	A	144.3
5	26/M	+	B	147.4
6	23/M	+	B	14494
7	23/M	+	A	119.0
8	24/M	+	AB	138.4
Mean ± SD				147.8 ± 18.2

Table 4. Platelet survival in patients with Korean hemorrhagic fever

Case No.	Age/Sex (yr)	Blood Group		Survival (hr)	
		Rh	ABO	Early	Later
1	23/M	+	O	138.0	171.5
2	23/M	+	B	100.1	197.7
3	23/M	+	A	126.8	173.2
4	22/M	+	A	125.0	154.2
5	23/M	+	A	89.5	157.2
6	23/M	+	A	109.4	150.4
7	24/M	+	A	109.5	167.6
8	23/M	+	B	136.0	138.8
9	23/M	+	A	113.8	136.9
10	23/M	+	B	136.9	154.8
11	24/M	+	O	70.1	155.8
12	24/M	+	A	87.6	128.0
Mean ± SD				111.9 ± 21.9	157.2 ± 18.8

and 58±17% in the early and later stages respectively. Two cases (Case 2 and 3) showed hypocellular marrow (21% and 25%) in the early stage (Fig. 2) whereas, in the later stage, hypercellular marrow (91%) was found in a patient (Case 4) whose initital marrow showed normal cellularity (44%) (Table 6).

The mean megakaryocyte number per 10^6 nucleated cells in the early stage was 5,204±1,786. The increase was statistically significant compared with 2,828±734 in control subjects ($p < 0.01$). The megakaryocyte number in the later stage was 3,808±1,504. This was not statistically different from the value in control subjects ($0.05 < p < 0.10$) or in the early stage ($0.10 < p < 0.20$)

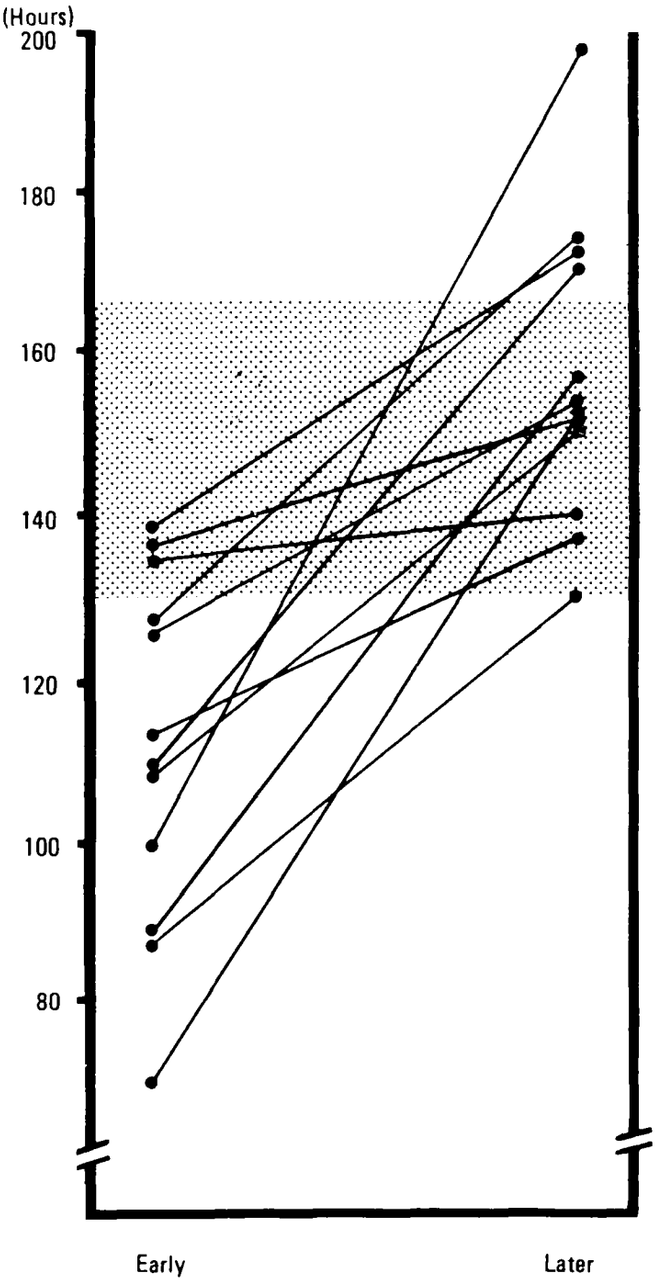

Fig. 1. Change of ^{111}In-labeled allogeneic platelet survival in patients with Korean hemorrhagic fever. Shaded area indicates mean±SD in normal controls.

(Table 5, 7, Fig. 3).

The corrected megakaryocyte number was 2,585±947 and 2,079±847 in the early and later stages respectively. The increase in the early stage was also significant compared with the corrected control value of 1,492±372 ($0.02 < p < 0.05$). The corrected megakaryocyte number in the later stage was not significantly different from the value in control subjects ($0.10 < p < 0.20$) or in the early stage ($0.20 < p < 0.50$) (Table 5, 7).

Table 5. Bone marrow findings in control subjects

Case No.	Age/Sex (yr)	Underlying Disease*	Cellularity (%)	Megakaryocyte	
				No**	Size (μm^2)
1	26/M	NHL (DH) IA	62	3,092 (1,917)	307.3
2	25/M	NHL (DH) IEB	53	2,214 (1,173)	330.1
3	27/M	NHL (NS) IA	43	3,772 (1,622)	288.9
4	21/F	NHL (DH) IIEA	51	3,206 (1,635	267.8
5	25/F	NHL (DH) IIEA	48	1,734 (832)	256.2
6	28/F	Nonspecific lymphadenitis	60	2,947 (1,768)	280.6
Mean±SD			53±7	2,828 (1,491 ±734 ±372)	288.5 ±26.9

* NHL: Non-Hodgkin's lymphoma, HD: Hodgkin's disease, DH: Diffuse histiocytic
NS: Nodular sclerosis

** Per 10^6 nucleated cells in bone marrow
Values in parentheses indicate corrected megakaryocyte numbers.

Table 6. Bone marrow cellularity in patients with Korean hemorrhagic fever

Case No.	Cellularity(%)	
	Early	Later
1	41	50
2	21	33
3	25	60
4	44	91
5	65	48
6	71	79
7	42	74
8	56	61
9	60	56
10	50	43
11	65	48
12	51	—
13	80	—
Mean±SD	52±17	58±17

Table 7. Number of megakaryocytes in patients with Korean hemorrhagic fever

Case No.	No. Megakaryocyte*			
	Early		Later	
1	4,766	(1,954)	3,544	(1,772)
2	6,702	(1,407)	6,790	(2,241)
3	4,689	(1,172)	5,024	(3,014)
4	4,860	(2,138)	1,569	(1,428)
5	6,501	(4,226)	3,114	(1,495)
6	3,865	(2,744)	5,268	(4.162)
7	10,101	(4,242)	2,493	(1,845)
8	3,170	(1,775)	4,289	(2,616)
9	4,382	(2,629)	4,090	(1,730)
10	4,788	(2,394)	3,449	(1,483)
11	3,572	(2,322)	2,261	(1,085)
12	5,549	(2,830)	—	(—)
13	4,706	(3,765)	—	(—)
Mean ±SD	5,204 ±1,786	2,585 ±947	3,808 ±1,504	2,079 ±847

* Per 10^6 nucleated cells in bone marrow. Values in parentheses indicate corrected megakaryocyte numbers.

The mean megakaryocyte size was 314.0±67.2 μm^2 and 301.1±48.3 μm^2 in the early and later stages respectively. The values were not significantly different from the control value of 288.5±26.9 um^2 ($0.20<p<0.50$ and $p>0.50$ respectively). No statistical significance was found between the two stages, as well ($0.20<p<0.50$). However, two cases (Case 5 and 7) showed larger megakaryocyte sizes than other patients while their megakaryocyte numbers were also higher than in other patients (Table 5, 8).

Positive correlations were found between the platelet count and platelet survival ($r=0.40$, $0.01<p<0.02$), and the megakaryocyte size and megakaryocyte number ($r=0.69$, $p<0.01$). In contrast, significant negative correlations were observed between the marrow cellularity and megakaryocyte number ($r=0.36$, $p<0.01$), and the megakaryocyte size and platelet survival ($r=-0.62$, $0.02<p<0.05$). When the corrected megakaryocyte number was compared with other parameters, the platelet count and platelet survival also revealed significant correlations to the corrected number (r

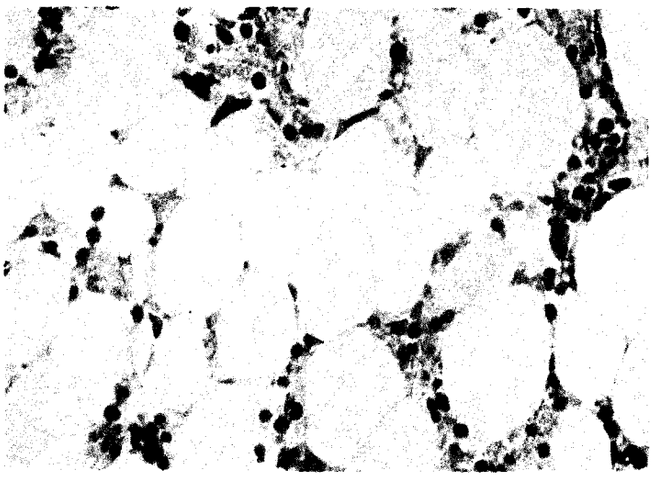

Fig. 2. Hypocellular marrow of Case 2 in the early stage of Korean hemorrhagic fever (PAS stain, ×200).

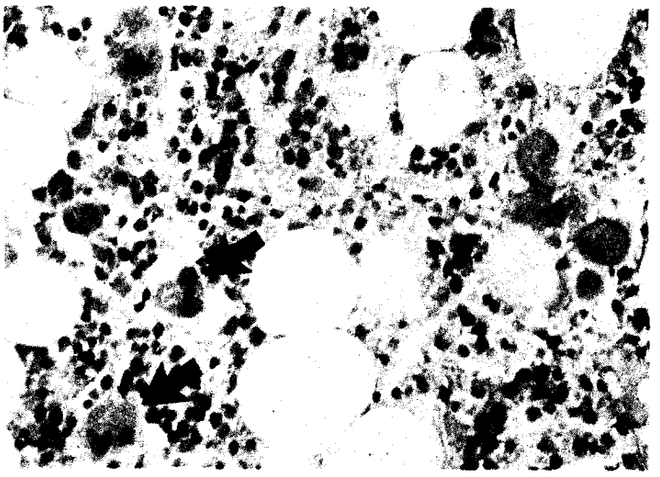

Fig. 3. Bone marrow of Case 7 showing a marked increase of megakaryocytes (arrows) in the early stage of Korean hemorrhagic fever (PAS stain, × 200).

$=0.20$, $p<0.02$ and $r=-0.39$, $p<0.01$, respectively) while the cellularity correlated directly to it ($r=0.59$, $p<0.01$). The megakaryocyte size also correlated significantly with the corrected megakaryocyte number ($r=0.79$, $p<0.01$)(Fig. 4).

DISCUSSION

Hemorrhagic manifestations in Korean hemorrhagic faver are impressive enough to have lead early investigators to name the disease after this feature. They are manifested by petechiae, echymoses, subconjunctival hemorrhage, epistaxis, hematemesis, hemoptysis, melena and hematuria (Sheedy et al. 1954; Lee et al. 1980). The pathogenetic mechanism of bleeding diathesis seen in this disease may generally be framed in terms of the following abnormalities; (a) thrombocytopenia, (b) disseminated intravascular coagulation, (c) abnormal platelet function, (d) capillary damamge, and (e) uremia.

Table 8. Megakaryocyte size in patients with Korean hemorrhagic fever

Case No.	Size (μm^2)	
	Early	Later
1	265.8	307.7
2	281.2	333.4
3	271.8	279.6
4	282.6	372.9
5	444.6	206.7
6	276.7	288.4
7	453.1	304.2
8	259.0	247.4
9	280.2	359.0
10	256.3	281.4
11	362.1	331.7
12	336.1	—
13	312.5	—
Mean ±SD	314.0 ±67.2	301.1 ±48.3

The most frequently observed abnormality in the coagulation profiles of Korean hemorrhagic fever is a depression of the platelet count. Almost all patients have a moderate to severe thrombocytopenia with platelet counts in the vicinity of 50,000/mm^3 during the early phases of the illness. Thrombocytopenia appears to be causally related to the bleeding phenomena observed during this period (Furth 1954).

Disseminated intravascular coagulation has been suggested as a cause of bleeding in Korean hemorrhagic fever since Dennis and Conrad(1968) reported a patient with accelerated intravascular coagulation. Although questions have been raised whether and how often, if any, disseminated intravascular coagulation occurs in these patients, well-designed studies from our laboratory (Lee et al. 1981) and from Soviet Union (Bunin and Abdurashitov 1976; Sirotin et al. 1977) revealed that it is encountered frequently during the initial days of the disease. However, the occurrence was transient and restored promptly as the disease progressed (Lee et al. 1981).

Functional defects of the platelets (Park and Lee 1982) and capillary damage (Barbero et al. 1953; Powell 1953) have also been reported in Korean hemorrhagic fever. The results of platelet function

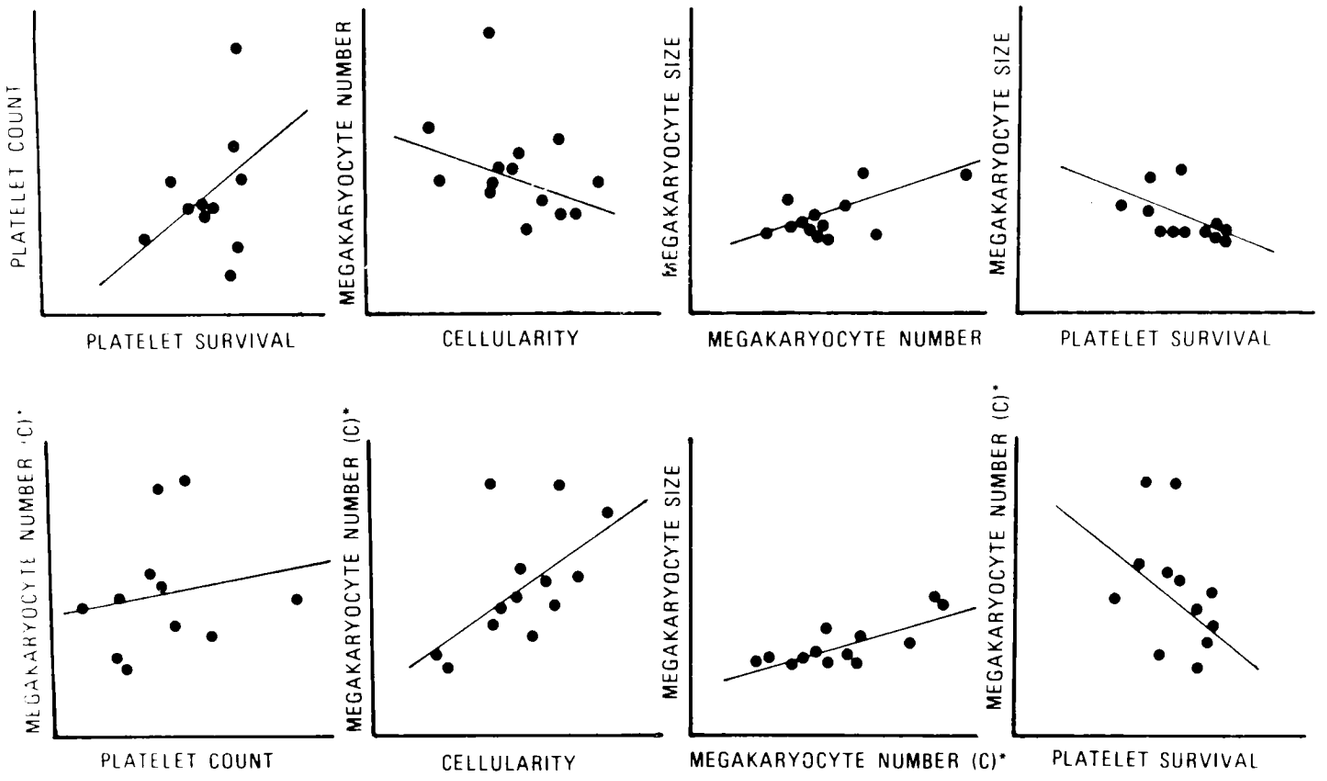

Fig. 4. Correlation between platelet survival and platelet count, bone marrow cellularity and megakaryocyte number, megakaryocyte number and megakaryocyte size, platelet surivival and megakaryocyte size, and corrected megakaryocyte number and platelet count, bone marrow cellularity, megakaryocyte size or platelet survival (C= Corrected).

studies, however, should be interpreted with caution considering the markedly low platelet counts in the majority of patients. Uremia, which is nearly always found in clinically apparent cases, may also be associated with hemorrhagic diathesis in later phases secondary to defective platelet function (Stewart and Castaldi 1967).

It seems likely that one or more of these factors, in some combinations, would contribute to the bleeding during each phase of the disease. Although there has been some controversy regarding the relative role of each factor described above, no one appears to argue against the major role of thrombocytopenia for the hemorrhagic manifestations observed in the early stage of the disease.

Concerning the cause of thrombocytopenia, a few possible explanations have been offered. Some insisted on accelerated destruction of platelets in the peripheral circulation (Paik et al. 1974; Moon et al. 1975; Lee et al. 1981) while others suggested the possibility of bone marrow failure, as well (Park et al. 1973; Chi et al. 1976; Hong et al. 1976; Lee and Lee 1979).

The study of platelet survival has proved of value in the investigation of the mechanism of thrombocytopenia and the quantitative evaluation of the factor(s) contributing to it. Prior to the development of ^{111}In-oxine, there was no suitable gamma-emitting label for tracing the platelets except ^{51}Cr-chromate the labeling efficiency of which was so low that large volumes of blood had been required to make it difficult to perform the procedure in thrombocytopenic and/or hypovolemic patients (The Panel on Diagnostic Application of Radioisotopes in Hematology, International Committee for Standardization in Hematology 1977).

^{111}In developed by McAfee and Thakur (1976a; 1976b) has many advantages over ^{51}Cr in the evaluation of platelet kinetics. The physical characteristics of ^{111}In include its half-life of 2.8 days and emission of gamma photons (173 and 247 kev; 89% and 94% abundant respectively). These characteristics are well suited for studies of the platelets. In addition, ^{111}In complexed with the lipid-soluble compound, 8-hydroxyquinoline (oxine), can be used to label platelets with high efficiency (70 to 90%). Hence, only a relatively small volume of blood is required to harvest an adequate

number of platelets for labeling (Thakur et al. 1976).

The platelets to be labeled in this study were harvested from an intermittent-flow centrifugation cell separator (Haemonetics 30 S) because a large amount of platelets was required to make several measurements at the same time. This procedure, together with ABO incompatibility of the allogeneic platelets, might have produced a little lower platelet survival. The mean platelet survival of 147.8 hours in normal controls is low compared with nine to ten days in other reports using ^{51}Cr or ^{111}In (Harker 1978; Heaton et al. 1979; Hawker et al. 1980; Heynes et al. 1980).

The way of evaluation in this study does not seem inappropriate, however, considering the facts that no significant differences in post-transfusion platelet increments are found even when donors are ABO incompatible, if HLA compatible, compared to those compatible in both systems (Lohrmann 1974) and that control subject and patients were tested with the same platelets. ABO types of the patients and normal controls did not appear to influence the platelet survival in this study.

In our patients, the platelet survival of the early stage was significantly depressed compared with the control values. The change between the early and later stages was also statistically significant.

Paik(1974) reported a moderate to marked reduction of the ^{51}Cr-labeled allogeneic platelet survival in mild and severe groups of Korean hemorrhagic fever patients respectively without comparison with the control values. In the mild group of patients whose mean platelet count was 64,600/mm^3, the platelet survival was 6.4 ± 0.30 days while the mean survival was 1.15 ± 0.37 days in the severe group with platelet counts in the vicinity of 18,600/mm^3. Considering the differences in methods of the platelet survival measurement and severity of the illness, our results seem to be in accordance with those of Paik (1974).

These findings indicate peripheral platelet loss or destruction in the early stage of Korean hemorrhagic fever. Reduced platelet survival also favors occurrence of disseminated intravascular coagulation in this period. Harker and Finch(1969) reported a marked reduction in platelet survival of patients with disseminated intravascular coagulation. The platelet survival of our patients was not so short as in these patients. The short duration and self-limiting course of disseminated intravascular coagulation in this disease may explain the less severe depression of platelet survival in our patients (Lee et al. 1981).

The platelet survival correlated directly with platelet counts suggesting a cause-and-effect relationship between the reduced platelet survival and thrombocytopenia. The platelet survival showed an inverse correlation with the megakaryocyte size and corrected megakaryocyte number suggesting some influence on the megakaryocyte production in bone marrow.

A compensatory increase in the number of megakaryocytes of bone marrow normally follows peripheral platelet loss or destruction (Harker 1968a; Harker and Finch 1969). If accelerated destruction of platelets and/or disseminated intravascular coagulation is the only cause(s) of thrombocytopenia observed in the early stage of Korean hemorrhagic fever, it should follow that the megakaryocyte number in bone marrow increases or, at least, remains normal (Craddock et al. 1955; Harker and Finch, 1969; Harker 1970). Trials to date, however, have shown conflicting results. Hyperplasia of megakaryocytes has been reported by some workers (Moon et al. 1975; Hullinghorst and Steer 1953; Lukes 1954; Lee et al. 1977; Whang and Lee 1979) while others pointed towards failure of thrombopoiesis in bone marrow as being involved in the pathogenesis of thrombocytopenia (Park et al. 1973; Chi et al. 1976; Hong et al. 1976; Lee and Lee 1979).

The controversy appears to have resulted from inadequate and unreliable methods of bone marrow examination for megakaryocytes. Most of the previous observations have been made on aspirated smears, but not on histologic sections. The results should be interpreted in relation to the stages of the illness as well because the changes in coagulation system is so rapid in this disease (Lee et al. 1981). Better informations would be expected if both the bone marrow examination and platelet kinetic studies are made simultaneously as in the present study.

Bone marrow megakaryocyte numbers were shown in our patients to be increased in the early stage of the disease when the platelet count was decreased and platelet survival was shortened. None of the cases revealed decreased number of megakaryocytes in the early stage. The megakaryocyte number was not depressed even in patients with hypocellular marrow.

The megakaryocyte number showed singificant correlations with the marrow cellularity and mega-

karyocyte size. The corrected megakaryocyte number correlated significantly not only with these two parameters, but also with the platelet count and platelet survival.

The bone marrow cellularity correlated inversely with the megakaryocyte number, but directly with the corrected number which is believed to approximate more closely to the total number of megakaryocytes in the body (Wickramasinghe 1975). These data are interpreted to suggest that megakaryocyte production is not so much affected by marrow depression as the other cell lines in the marrow even though the total thrombopoietic activity might have been affected to some degree in occasional patients with hypocellular marrow.

These findings together with the increase of megakaryocyte number in the early stage of Korean hemorrhagic fever indicate that megakaryocyte production was largely affected by the numerical and kinetic changes of the platelets in the peripheral circulation and that marrow production of the megakaryocytes was relatively optimal and thrombopoietic failure was not, if any, the major cause of the profound thrombocytopenia in the early stage of Korean hemorrhagic fever.

In Korean hemorrhagic fever, Moon et al. (1975) showed that the mean megakaryocyte diameter measured on aspirated smears was longer in early stages than in late stages. However, the results were not compared with those in control subjects. In the present study, the megakaryocyte size was measured from the longest and shortest diameters, and the mean size of 288.5 μm^2 in controls compared favorably with a normal mean size of 300 μm^2 in other series (Thiele et al. 1983). The megakaryocyte size of our patients did not show, in either stage of the disease, significant difference from the mean value in control subjects. The change between the early and later stages also was not statistically significant. This is not consistent with the previous report of Moon et al.(1975) and with the finding of a usual increase in the megakaryocyte volume in patients with a reduction of platelet survival and/or thrombocytopenia (Moon et al. 1975; Harker and Finch 1969; Harker 1968; Ebbe et al. 1968). While it may be due to the relatively mild reduction of the platelet survival in our patients, the two cases showing larger mean megakaryocyte sizes together with much higher values for megakaryocyte numbers suggest that an increase in megakaryocyte size might be observed to some degree in patients with lower platelet survival and/or more abundant megakaryocytes in bone marrow. This view is also supported by the inverse correlation between the megakaryocyte size and platelet survival.

All these findings, together with the appearance of frequent giant platelets in the peripheral circulation (Moon et al. 1975), argue against the possibility of thrombopoietic failure in patients with Korean hemorrhagic fever.

The bone marrow cellularity of most cases was in normal range both in the early and the later stages of the disease. In a small proportion of patients, however, the bone marrow sections showed hypocellular marrow in the early stage restoring to normal in the later stage. These cases and another patient revealing hypercellular marrow in the later stage, suggest the possibility that, in the early stage of Korean hemorrhagic fever, bone marrow depression occurs albeit mild and that thrombocytopenia may be related to some degree to the bone marrow failure to increase effective thrombopoiesis sufficiently to compensate for the decreased platelet survival. Also raised from these findings are the following questions. Might the megakaryocyte number have increased to a greater degree if not this possible bone marrow depression? Can it explain the megakaryocyte size not increased to the degree as might be expected in usual patients with decreased platelet survival and/or thrombocytopenia? The answers may provide the key to explain the profound depression of platelet counts in comparison with the relatively mild reduction of platelet survival in the early stage of Korean hemorrhagic fever as shown in this study.

We suggest reasonable assessment of current data must lead to the conclusion that thrombocytopenia observed in the early stage of Korean hemorrhagic fever is mainly derived from increased consumption of platelets in the peripheral circulation. Significant correlations between each parameter of the megakaryocyte-platelet axis indicated the cause-and-effect relationship between the decresased platelet survival resulting in thrombocytopenia and the compensatory production of megakaryocytes in bone marrow.

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= 국문초록 =

한국형 출혈열에 있어서 Thrombokinetics에 관한 연구

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한국형 출혈열 환자의 주증상의 하나인 출혈성 경향을 초래하는 혈액응고계의 변화 중 가장 흔히 관찰되고 또한 중요한 역할을 할 것으로 믿어지는 혈소판 수의 감소 기전을 규명하기 위하여 13명의 초기환자를 대상으로 발병 초기와 진행 후의 혈소판수 및 혈소판 수명 측정과 골수 검사를 시행하여 다음과 같은 성적을 얻었다.

1. 혈소판수는 초기에는 54,000±28,000/mm^3으로 감소하였으나, 후기에는 245,000±82,000/m^3으로 증가하였다($p<0.01$).

2. ^{111}In-oxine을 이용한 동종 혈소판수 명은 초기에는 111.9±21.9 시간으로 정상 대조군의 147.8±182 시간에 비해 유의하게 감소하였다($p<0.01$). 후기에는 157.2±18.8 시간으로 정상 대조군과 통계적으로 유의한 차이가 없었으나($p>0.20$) 초기에 비해서는 유의하게 증가하였다 ($p<0.01$).

3. 골수조직의 세포충실도는 초기 및 후기에 각각 평균 52±17%와 58±17% 였으나 초기의 환자 중에는 각각 21%로 감소된 예가 2례 관찰되었고, 후기의 1례에서는 91%로 증가된 것을 보여주었다.

4. 골수조직내 유핵세포 10^6개중 거대핵세포의 수는 한국형 출혈열 초기에는 5,204±1,786으로 대조군 2,828±734에 비해 유의하게 증가하였으나($p<0.01$), 후기에는 3,808±1,504로 유의한 차이가 없었고 ($0.05<p<0.10$), 전후기간의 변화도 통계적으로 유의하지 않았다 ($0.10<p<0.20$).

5. 골수내 거대핵세포의 면적은 발병 초기와 후기에 각각 314.0±67.2 μm^2과 301.1±48.3 μm^2로 대조군의 288.5±26.9 μm^2에 비해 유의한 차이가 없었으며 (각각 $0.20<p<0.50$ 및 $p<0.50$). 전후기 간에도 역시 유의한 변화를 보이지 않았다 ($0.20<p>0.50$)

그러나 초기의 2례에서는 거대핵세포의 면적이 각각 평균 444.6 μm^2 및 453.1 μm^2로 증가되어 있었으며 이들에서는 유핵세포 10^6개중 거대핵세포의 수도 각각 6,501 및 10,101로 다른 환자들에서보다 증가되어 있었다.

6. 발병 초기에는 혈소판 수명, 혈소판수, 거대핵세포수 및 거대핵 세포면적 사이에 유의한 상관관계가 관찰되었다.

이상의 성적에서 한국형 출혈열 초기에 관찰되는 혈소판수의 감소는 주로 혈소판의 소모증가에 기인하는 것을 알 수 있었다.