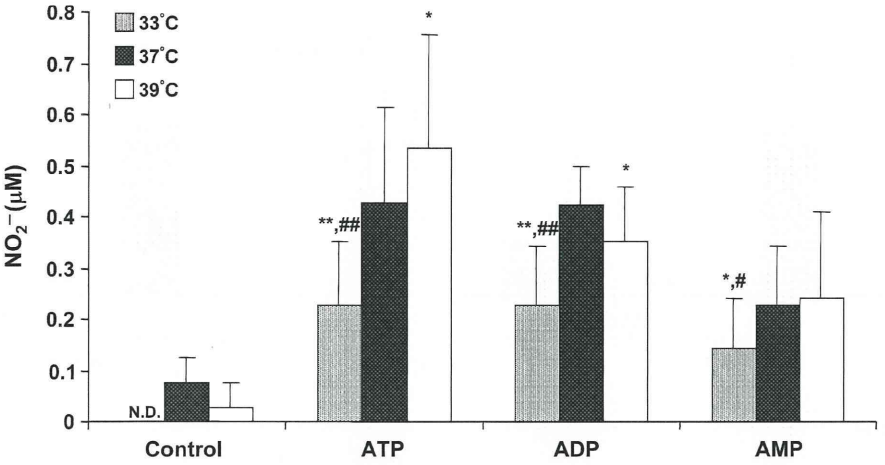

Fig. 4 Effects of incubation temperature on NO production by ATP-, ADP-, and AMP-stimulated microglia. Microglia (4×10^4 cells/well) were cultured with or without 1 mM ATP, ADP, or AMP at 33, 37, and 39°C for 6 h. Concentrations of NO_2^- in culture supernatants were measured by colorimetric assay with Griess reagent. Data are expressed as mean ± SD ($n = 4$). *N.D.* not detected. $^*P < 0.05$, $^{**}P < 0.01$ compared with 37°C. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared with 39°C

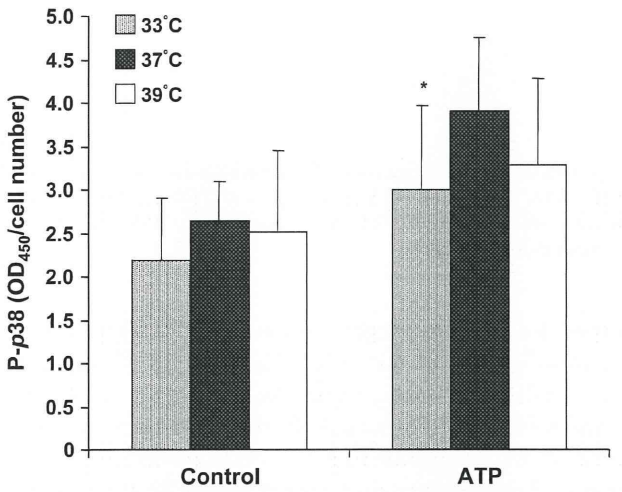

Fig. 5 Effects of incubation temperature on P-*p*38 in ATP-stimulated microglia. Microglia (4×10^4 cells/well) were cultured with or without 1 mM ATP at 33, 37, and 39°C for 1 min. P-*p*38 in cultured microglia was examined using a Fast activated cell-based ELISA kit. Data are expressed as mean ± SD ($n = 6$). $^*P < 0.05$ compared with 37°C

responses to temperature alteration using ATP, an intrinsic microglial activator.

The finding that a lower temperature reduced ATP-activated microglial release of pro-inflammatory cytokines and NO is in accordance with results from other studies that used LPS stimulation [14–17]. In in vivo studies, therapeutic hypothermia also attenuates the increase in levels of these substances in the central nervous system after brain injury [4, 12, 30], and this is associated with a favorable outcome, compared with normothermia [4, 12]. Therefore, the present findings strongly support the idea that the reduction of microglial production of pro-inflammatory cytokines and NO is important in the neuroprotective effects of hypothermia. Moreover, our findings suggest that this benefit is largely related to the attenuation of the early phase inflammatory response of microglia.

In addition, brain temperature has been recognized as a crucial factor in the development of neuronal damage. Mild hypothermia (<35°C) alleviates neuronal damage induced by ischemia and trauma [31, 32], whereas hyperthermia (>39°C) exacerbates such damage [32]. Temperature elevations in the brain are often observed in patients with severe head injury [24]. Therefore, the present temperature-dependent changes in microglial TNF-α and NO production imply that monitoring of these substances in CSF during the early phase might be useful as biomarkers for responses to hypothermia-related neuronal protection and hyperthermia-related neuronal injury.

Compared with LPS stimulation [17], ATP activation of microglia for 6 h caused a different pattern of NO production at 39°C: NO level at 39°C was increased in comparison with 37°C, whereas this difference was not observed in LPS-activated cells. Although incubation temperature was the main factor affecting the production of mediators, it is plausible that this difference actually resulted from the interaction between both the temperature and the stimulator used, and that this determined the pattern of mediator production. When ATP activates microglia, temperature elevation (up to 39°C) and the production of some inflammatory factors are likely to develop in parallel since temperature-dependent changes were seen not only in NO, but also in TNF-α production. However, this was not

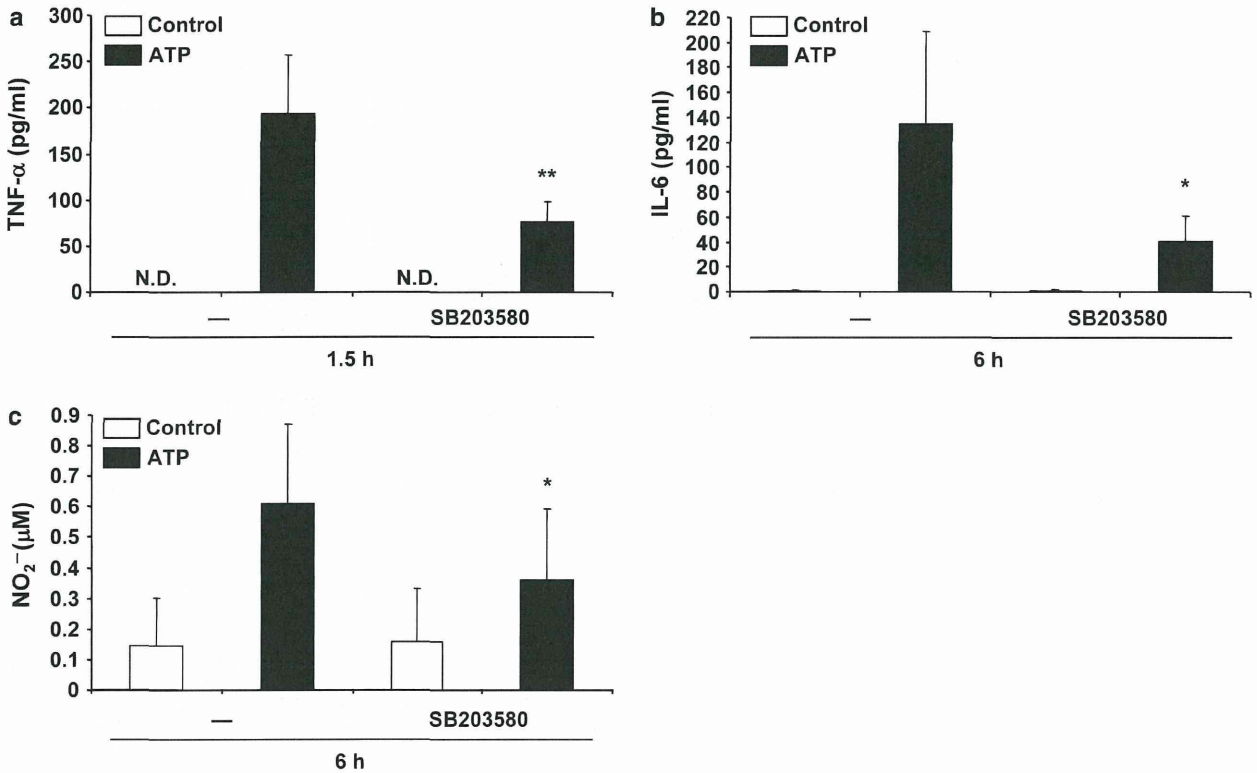

Fig. 6 Effects of *p*38 inhibitor on TNF-α, IL-6, and NO production by ATP-stimulated microglia. Microglia (4×10^4 cells/well) were cultured with or without 1 mM ATP in the presence or absence of a *p*38 inhibitor (SB303580; 10 μM) for 1.5 h (TNF-α) or 6 h (IL-6 and NO_2^-) at 37°C. Concentrations of TNF-α (**a**), IL-6 (**b**), and NO_2^- (**c**) in culture supernatants were measured using ELISA (**a**, **b**) and a colorimetric assay with Griess reagent (**c**). Data are expressed as mean ± SD ($n = 5$ for **a** and **c**, and $n = 6$ for **b**). *N.D.* not detected. $^*P < 0.05$, $^{**}P < 0.01$ compared with ATP alone

the case for IL-6, another inflammatory factor, whose production was reduced at 39°C in accordance with previous results using LPS [17]. IL-6 has generally been considered potentially neurotoxic and linked to exacerbation of brain injury [1, 5, 7]. However, it is also thought to have a neuroprotective role by inducing neurotrophic factors [33]. We thus speculate that the beneficial effects of IL-6 on neurons might be reduced in hyperthermic states, whereas its detrimental effects might be inhibited by hypothermia. Further investigations are necessary to clarify how LPS and ATP stimulation may cause different patterns of NO production at 39°C.

This increased production of TNF-α and NO at 39°C is a novel finding. Hasday's group demonstrated that exposure to temperatures of 38.5–40°C reduces LPS-stimulated TNF-α expression in monocytes/macrophages [34–36], while increasing IL-1β expression in macrophages [34]. NO production in macrophages was enhanced by increasing culture temperature, but only when the temperature reached the level of heat shock (41°C) [37]. At 43°C, LPS-stimulated TNF-α expression in macrophages was also reduced, while IL-1β expression was not altered [34]. In addition, another study showed that LPS-stimulated IL-6 expression in macrophages was unchanged at temperatures up to 40°C [35], but was reduced at 42°C [38]. Combining our previous [17] and present studies together, IL-6 production of both LPS- and ATP-stimulated microglia was reduced at 39°C. These results indicate the following four points. First, temperature elevation (38.5–40°C) might exert different effects on cytokines in a specific manner. Second, temperature elevation and heat shock (41–43°C) might regulate the expression of cytokines and/or NO through distinct mechanisms. Third, microglia and monocytes/macrophages might exhibit different susceptibility to temperature elevation. Fourth, as mentioned above, temperature elevation might affect a stimulus-dependent signaling pathway, leading to a distinct cytokine/NO production pattern.

Temperature Reduction and *p*38-Mediated Cytokines/NO Production

We showed that ATP activates *p*38 in microglia, and that lowering of the temperature rapidly suppresses this activation, compared to 37°C. Moreover, *p*38 inhibition attenuated TNF-α, IL-6, and NO production from ATP-

activated microglia, confirming that *p*38 regulates production of these mediators. These result, and the fact that suppression of *p*38 activation was followed by reduced production of pro-inflammatory cytokines and NO, indicate that temperature reduction attenuates the release of these inflammatory factors, at least in part, via suppression of *p*38 in ATP-activated microglia. Thus, it appears that microglial *p*38 is one key cellular target by which therapeutic hypothermia achieves its neuroprotective effects. We are aware, on the other hand, that other members of the mitogen-activated protein kinase family, namely, extracellular signal-regulated protein kinase (ERK) and c-Jun N-terminal activated protein kinase (JNK) are also implicated in the inflammatory response [25, 39]. However, activation of *p*38 is more rapid than that of ERK and JNK [23, 40], and *p*38 is an important signal transduction molecule that contributes to glia-induced neuronal death [40]. Thus, there is great interest in the inhibition of this kinase in therapeutic research. In contrast, elevated temperature did not affect P-*p*38 in this study, which is in accordance with a previous study done in macrophages [34]. The fact [34] that temperature elevation can increase the expression of a cytokine by enhancing recruitment of nuclear factor (NF)-κB to its gene promoter might explain for the increased production of TNF-α and NO seen at the present elevated temperature. Further studies focusing on NF-κB are required to assess this relationship.

Incubation Temperature and ATP Metabolite-Induced Cytokines/NO Production

Since ATP is easily metabolized to ADP and AMP, it is conceivable that these metabolites contribute to the effects of ATP-induced mediator production from microglia. TNF-α production was stimulated less by ADP than by ATP at all temperatures, with similar temperature-dependent alterations. Moreover, we found that there was greater induction in NO production by ATP and ADP than by AMP, although there were no statistical differences among the stimuli at each temperature. In addition, the reduction in NO production at 33°C, compared with 37 and 39°C, was also observed in ADP- and AMP-stimulated microglia. Taken together, these results indicate that the changes in TNF-α and NO production in response to incubation temperature in ATP-activated microglia are mediated, at least in part, by the effects of ADP and AMP. To our knowledge, this is the first report to show that these ATP metabolites stimulate the production of TNF-α and NO from microglia.

Incubation Temperature and IL-10 Production

IL-10 production by ATP-stimulated microglia was low and unchanged at all three temperature conditions in the early phase (6 h). In contrast, with LPS-activated microglia, the increase in IL-10 level was reduced at 33°C, but augmented at 39°C after 24–72 h [17]. LPS stimulation of monocytes has been found to initially lead to synthesis of pro-inflammatory cytokines, and later to the synthesis of IL-10 [41]. These findings suggest that increased IL-10 production is susceptible to the effects of temperature alteration at the late phase. However, we were unable to focus on this period because of the possibility of ATP degradation.

Limitations of the Study

We were unable to determine in vivo levels of the cytokines and NO during hypothermia, normothermia, and hyperthermia, so the clinical significance of the present results remains to be determined. In addition, the results might have yielded more clinically relevant data had the activated microglia been removed directly from the injured brain, or had human microglia been used. These types of studies would be of great interest for confirming the clinical significance of the present findings.

Conclusions

This study shows for the first time the microglial responses to temperature alteration using an intrinsic microglial activator, ATP, which is released after brain insults. Lowering of the temperature rapidly reduced *p*38 activation and the subsequent *p*38-regulated production of TNF-α, IL-6, and NO in ATP-activated rat microglia. Our results suggest that the reduction of early phase inflammatory responses via suppression of *p*38 in microglia is one possible neuroprotective mechanism of therapeutic hypothermia. Temperature elevation increased TNF-α and NO production in the rat microglia. The observed temperature-dependent changes imply that monitoring of the TNF-α and NO levels in CSF during the early phase might be useful as biomarkers for responses to therapeutic hypothermia and hyperthermia.

Acknowledgments This research was supported in part by grants from the Ministry of Education, Culture, Sports, Science, and Technology of Japan, Grant-in-Aid for Young Scientists (B), No. 20791077 to T. M.

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Therapeutic Apheresis and Dialysis

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Guidelines

2011 Japanese Society for Dialysis Therapy Guidelines for the Treatment of Hepatitis C Virus Infection in Dialysis Patients

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[1]Working Group for the Preparation of Guidelines for the Treatment of Hepatitis C Virus Infection in Dialysis Patients, [2]Chairman, Subcommittee for the Preparation of Guidelines, [3]Chairman, Scientific Committee, and [4]Chairman, Board of Directors, Japanese Society for Dialysis, Tokyo, Japan

INTRODUCTION

Objectives of the preparation of the guidelines

The prevention, diagnosis, and treatment of hepatitis C Virus (HCV) infection are clearly important for the management of patients undergoing chronic hemodialysis, because (i) the HCV infection rate is high in dialysis patients; (ii) the outcome is poorer in HCV-infected than non-infected dialysis patients; and (iii) an improvement in the outcome can be expected by the prevention or diagnosis and treatment of HCV infection. Therefore, it was decided to prepare "guidelines for the treatment and management of hepatitis C at dialysis facilities by dialysis physicians and nephrologists in cooperation with hepatologists" by the instruction of Tadao Akizawa, Chairman of the Board of Directors of the Japanese Society for Dialysis Therapy, and Hideki Hirakata, Chairman of the Scientific Committee, and under the leadership of Tadashi Tomo, Chairman of the Committee for the Preparation of the Guidelines. In preparing the guidelines, it was agreed (i) that they would be applied to chronic dialysis patients; and (ii) that they would be used by physicians at dialysis facilities. They would also be prepared to inform hepatologists about the dose of interferon and the criteria for the introduction and reduction of interferon administration in dialysis patients. Their preparation was initiated at the first meeting of the Committee for the Preparation of Guidelines for the Treatment of Hepatitis C Virus Infection in Dialysis Patients on 6 January 2009.

Environment and history of the preparation of the guidelines

Prior to this, in April 2008, the Kidney Disease: Improving Global Outcomes (KDIGO) group presented the "KDIGO Clinical Practice Guidelines for the Prevention, Diagnosis, Evaluation, and Treatment of Hepatitis C in Chronic Kidney Disease" as the first guidelines by the KDIGO itself in *Kidney International* (1). The guidelines were a 107-page tour de force consisting of five chapters dealing with (i) detection and evaluation of HCV in CKD patients; (ii) treatment of HCV-infected CKD patients; (iii) prevention of HCV infection in the dialysis room; (iv) treatment of HCV infected patients before and after kidney transplantation; and (v) diagnosis and treatment of HCV-related retinopathy, were compiled under the supervision of Michel Jadoul and David Roth, and described the diagnosis, treatment, and prevention of HCV infection in patients with CKD in the maintenance period, dialysis patients, and patients undergoing kidney transplantation. The ISN informed its members of these guidelines and recommended to apply them in consideration of the state of each country, region, and facility (implantation), because

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they contained provisions not necessarily based on strong evidence.

Thus, the Working Group for the Preparation of the Guidelines for the Treatment of Hepatitis C Virus Infection decided to make the guidelines cover the (i) diagnosis, (ii) treatment, and (iii) prevention of HCV infection in dialysis patients, and (iv) their management before and after transplantation on the basis of the items of the KDIGO guidelines by securing the cooperation of experts in dialysis and HCV hepatitis. In addition, as the aminotransferase levels are low in dialysis patients, and as the method for the assessment of fibrosis was not established, some members considered it necessary to include test methods and diagnostic criteria, and the guidelines were decided to comprise five chapters dealing with (i) screening, (ii) management (methods and frequencies of blood tests and imaging studies), (iii) indications of antiviral therapies, (iv) treatment by antiviral therapies (including patients expected to receive kidney transplantation), and (v) prevention of HCV infection at hemodialysis facilities.

The references consisted primarily of English and Japanese literature published by the end of 2008, but domestic and overseas guidelines were also included.

Committee members involved in the preparation of the guidelines

Tadao Akizawa, Chairman, Board of Directors, Japanese Society for Dialysis Therapy
Hideki Hirakata, Chairman, Scientific Committee, Japanese Society for Dialysis Therapy
Tadashi Tomo, Chairman, Subcommittee for the Preparation of Guidelines of the Japanese Society for Dialysis Therapy
Working Group for the Preparation of Guidelines for the Treatment of Hepatitis C Virus Infection in Dialysis Patients

Chairman	Takashi Akiba (Tokyo Women's Medical University)
Vice-chairman	Kazuhiko Hora (Hokushin General Hospital)
Members	Michio Imawari (Showa University)
	Chifumi Sato (Tokyo Medical and Dental University)
	Eiji Tanaka (Shinshu University)
	Namiki Izumi (Musashino Red Cross Hospital)
	Takashi Harada (Nagasaki Kidney Hospital)
	Ryoichi Ando (Musashino Red Cross Hospital)
	Kan Kikuchi (Tokyo Women's Medical University)

All members listed above have submitted a conflict of interest disclosure report to the General Affairs Committee.

Times and dates of meetings of the Committee for the Preparation of Guidelines for the treatment of hepatitis C virus infection in dialysis patients

1st Meeting	6 January 2009	18:00–20:00	Seiyoken, Nihonbashi
2nd Meeting	17 June 2009	18:00–20:00	Seiyoken, Nihonbashi
3rd Meeting	30 September 2009	18:00–20:00	Seiyoken, Nihonbashi
4th Meeting	25 December 2009	18:00–20:00	Seiyoken, Nihonbashi
5th Meeting	5 February 2010	18:00–20:00	Seiyoken, Nihonbashi
6th Meeting	4 June 2010	18:00–20:00	Seiyoken, Nihonbashi
55th Consensus Conference on Hepatitis C, Scientific Committee, Japanese Society for Dialysis Therapy	20 June 2010	13:30–16:30	Kobe International Conference Center, 1st Conference Room
7th Meeting	6 August 2010	18:00–20:00	Seiyoken, Nihonbashi
Public Hearing	16 January 2011	13:00–15:00	Clinical Lecture Hall, Tokyo Women's Medical University
8th Meeting	4 February 2011	18:00–20:00	Office Tokyo, 4F, Meeting Room A4

Evaluation of the evidence and recommendation levels

The evidence and recommendation levels were prepared on the basis of the position paper "Grading evidence and recommendations for clinical practice guidelines in nephrology" (2) issued by KDIGO in 2006 and the Working Group Report on the Grading of Evidence Levels and Degrees of Recommendation disclosed by the Japanese Society for Dialysis Therapy on 16 November 2009 (Table 1) (later published in the *Journal of the Japanese Society for Dialysis Therapy* with modifications) (3).

TABLE 1. *Working Group Report on the grading of evidence levels and degrees of recommendation, 16 November 2009*

Chairman of WG: Masashi Fukagawa
Members of WG: Kazutaka Kukita, Yusuke Tsukamoto, Tsubakihara Yoshiharu, Yoshizo Kaizu, Eiji Kusano, Masaaki Nakayama
Chairman, Subcommittee for the Preparation of Guidelines: Tadashi Tomo
Chairman, Scientific Committee: Hideki Hirakata

General Principles
(1) Considering the situation that various global and local guidelines have been issued, the following general principles are observed.
(2) The consistency of the style of the text of the guidelines will be evaluated in the future.
(3) After the report is submitted to and approved by the Board of Directors, its details will be published formally as a WG Report in the Journal of Japanese Society for Dialysis Therapy.

On the evaluation of evidence levels
(1) Basically, the current evidence grading method of KDIGO is followed (Kidney International, 2006, see the attached table).
(2) The following may be decided by the responsibility of the working group for each guideline. However, the criteria and reasons must be stated clearly.
  (a) Restriction of conditions for the adoption of research papers (size, period, etc.)
  (b) Upgrading and downgrading of evidence (depending on the situation, that the data are about Japanese subjects may be regarded as a condition of upgrading).
(3) Papers in Japanese may be adopted by the judgment of the WG if the evidence level can be evaluated.
  (a) If they are adopted, the reason for the adoption and the evaluation of the evidence level must be stated clearly.
  (b) Maximum support for publication in English must be provided until the Guidelines are published in English.
(4) Abstracts are not adopted, in principle.

On the recommendation level
(1) Graded into 2 levels (strong, weak)
(2) The following expressions are used.
  (a) It is recommended to . . . , It is recommended not to . . . (strong)
  (b) It is desirable to . . . , It is desirable not to . . . (weak)
  (c) Since negative sentences such as "It is disrecommended to . . . or it is undesirable to . . ." is a strong expression, "It is recommended not to . . . or it is desirable not to . . ." is used by attaching conditioning modifications such as "as a routine procedure".
(3) Ungraded expert opinions may be attached to items lacking evidence. In this instance, only those agreed on by two thirds or more of the WG members are adopted.

Table of abbreviations

AFP	α-fetoprotein
ALT	alanine aminotransferase
AST	aspartate aminotransferase
AUC	area under the plasma concentration time curve
Ccr	creatinine clearance
Cmax	peak serum concentration of a therapeutic drug
EIA	enzyme-linked immunosorbent assay
EOB-MRI	EOB-magnetic resonance imaging
EPO	erythropoietin
ESA	erythropoiesis stimulating agent
HA	hyaluronate
HCV	hepatitis C virus
IFN	interferon
KDIGO	Kidney Disease: Improving Global Outcomes
NIDDM	non-insulin-dependent diabetes mellitus
PCR	polymerase chain reaction
PEG-IFN	pegylated interferon
PIVKA-II	proteins induced by vitamin K absence-II
PLP	pyridoxal-5′-phosphate
PNALT	persistent normal ALT
ROC curve	receiver operating characteristic curve
RT-PCR	reverse transcriptase PCR
RVR	rapid virological response
SNMC	stronger neo-minophagen C
SVR	sustained virological response
Tmax	maximum drug concentration time
TRX	thioredoxin
UDCA	ursodeoxycholic acid
VRAD	virus removal and eradication by double filtration plasma pheresis

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SCREENING OF DIALYSIS PATIENTS FOR HEPATITIS C VIRUS INFECTION

[Statements]

1. The serum aminotransferase levels are lower in dialysis patients than in individuals with normal renal function. (Evidence level: High, Recommendation level: Strong)
2. The serum aminotransferase levels are higher in HCV-antibody-positive than in negative dialysis patients, but the criteria for the general population cannot be applied to dialysis patients. (Evidence level: High, Recommendation level: Strong)
3. In dialysis patients, it is desirable to measure the serum aminotransferase levels at least once a month even if they are asymptomatic. (Evidence level: Low, Recommendation level: Weak)
4. It is recommended to perform the HCV antibody test and, if necessary, the HCV-RNA test at the

introduction of dialysis and the acceptance of patients. (Evidence level: Low, Recommendation level: Strong)
5. In dialysis patients, it is desirable to perform the HCV antibody test at least once every 6 months even if HCV antibody is negative on the initial test. (Evidence level: Low, Recommendation level: Weak)
6. If the serum aminotransferase level increases with no clear cause, it is recommended to perform an ad hoc HCV-RNA or HCV core antigen test in addition to the HCV antibody test. (Evidence level: Low, Recommendation level: Strong)
7. If an HCV-positive patient considered to be due to nosocomial infection that has been detected, it is recommended to perform the HCV-RNA or HCV core antigen test in all dialysis patients who may have been exposed. (Evidence level: Very low, Recommendation level: Strong)

[Comments]

1. The serum aminotransferase level is lower in dialysis patients than in individuals with normal renal function. (Evidence level: High, Recommendation level: Strong)

Serum aminotransferase levels (AST, ALT) as indices of liver function have been reported to be lower in dialysis patients than in individuals with normal kidney function. There has been a report that the ALT level was 15.6 ± 12 IU/L in dialysis patients and 22.7 ± 18 IU/L in normal controls and that the upper limit of the normal range of ALT in dialysis patients was 27 IU/mL (1). Thus, if the upper limit of the normal range was set at 25 IU/L, then the ALT level was normal in 67% of dialysis patients (2). There is also a report that the AST levels in healthy individuals and dialysis patients were 22.3 (22.0 ± 22.7) and 20.6 (21.6 ± 23.6), respectively, that the ALT levels were 20.3 (19.9 ± 20.7) and 16.3 (15.3 ± 17.3), respectively, and that the cutoff values effective for the prediction of HCV infection were 18 for AST and 16 for ALT (3). Since the serum aminotransferase levels are lower in dialysis patients than the standards in the general population, their cutoff values for the prediction of HCV infection should be set at lower levels in these patients. It has been known that the serum aminotransferase levels in uremic patients are low and negatively correlate with the blood urea nitrogen level (4), and factors that inhibit the serum aminotransferase activities have been reported to accumulate in patients' serum with elevations of the serum aminotransferase levels due to dialysis (5). However, the level of pyridoxal-5'-phosphate (PLP) is positively correlated with the AST and ALT levels. Additionally, serum aminotransferase levels were significantly lower in the PLP-deficient group than in the normal group, being 9.2 ± 0.3 vs. 13.4 ± 0.7 for AST and 8.6 ± 0.6 vs. 11.4 ± 0.9 for ALT. Also, as the AST and ALT levels were elevated by supplementation of PLP only in the PLP-deficient group, deficiency of PLP, which acts as a coenzyme of aminotransferases, has been suggested to partly explain the low aminotransferase levels in dialysis patients (6). There is also a report that, in uremia, the enzyme activity of PLP is lost as its lysine-binding site is carbamylated by cyanogen salts formed by urea (7). In contrast, it has also been reported that the Vitamin B6 and PLP levels are normal in dialysis patients and thus, the low serum aminotransferase levels cannot be explained by Vitamin B6 deficiency (8,9).

Therefore, based on the clinical observations to date and abnormalities of enzyme activities in uremic patients, serum aminotransferase levels are considered to be lower in dialysis patients than in people with normal kidney function.

2. The serum aminotransferase levels are higher in HCV-antibody-positive than in negative dialysis patients, but the criteria for the general population cannot be applied to dialysis patients. (Evidence level: High, Recommendation level: Strong)

The serum aminotransferase levels are normal in dialysis patients regardless of whether they are negative or positive for HCV antibody. However, the ALT level is higher in HCV antibody positive dialysis patients than in HCV antibody negative dialysis patients (2.7 ± 20.0 and 12.5 ± 8.8, respectively) (10). Particularly, the simultaneous detection of HB antigen and HCV-RNA has been related to ALT elevation. Also, it has been reported that the ALT level was 32.4 ± 24.2 and 33.7 ± 27.2 in male and female HCV-antibody-positive dialysis patients, respectively, but 17.0 ± 11.4 and 13.9 ± 6.1 in male and female HCV-antibody-negative patients, respectively. The ALT level was also reported to be higher in HCV-RNA-positive than in HCV RNA negative patients. However, the ALT level was not related to the HCV genotype (11). In HCV-antibody-positive, HCV-antibody-negative, HCV-RNA-positive, and HCV-antibody-negative dialysis patients, the ratio of ALT/upper limit of the normal range was 0.77 ± 0.57, 0.38 ± 0.23, 0.81 ± 0.57, and 0.37 ± 0.23, respectively. The cutoff value of ALT for being HCV-antibody-positive as determined

from the receiver operating characteristic (ROC) curve was 50% of the upper limit of the normal range (sensitivity: 67%, specificity: 83%) and that for being HCV-RNA-positive was 45% (sensitivity: 71%, specificity 80%). Also, the observed value/upper limit of normal range of ALT was clearly higher in HCV-RNA-positive than in HCV-RNA-negative dialysis patients (12). Moreover, this value was reported not to differ in the group without hepatitis but to be higher in the group with hepatitis compared with the group without hepatitis, suggesting that the ALT level of HCV-RNA-positive dialysis patients may be useful as a marker of liver disorder obtained by liver biopsy (13). However, histological findings obtained by liver biopsy were reported to be milder, and the ALT level to be lower, in HCV-positive dialysis patients than in HCV-positive individuals with normal kidney function (14,15).

Therefore, the serum aminotransferase levels are considered to be higher in HCV-antibody-positive dialysis patients than in those negative, but the criteria for the general population are not considered to be applicable to dialysis patients.

3. In dialysis patients, it is desirable to measure the serum aminotransferase levels at least once a month even if they are asymptomatic. (Evidence level: Low, Recommendation level: Weak)

While there is no evidence concerning the frequency of measurement of the serum aminotransferase levels in dialysis patients, there have been reports that the serum aminotransferase levels and the ratio of ALT/upper limit of the normal range has been reported to be higher in HCV-antibody-positive and HCV-RNA-positive patients than in negative patients (10–12,16). Although the ALT level was elevated in only 51% of the HCV-RNA-positive patients after kidney transplantation, but that the ALT level was correlated with the degree of liver tissue damage evaluated by liver biopsy, and that ALT can serve as a marker of liver tissue damage in HCV-RNA-positive recipients of kidney transplantation (13). Therefore, observation of changes in ALT levels by regular examinations may lead to the early detection of HCV infection, and the possibility of HCV infection must always be considered even if the serum aminotransferase levels are within the normal ranges.

Liver function tests are usually performed once a month in dialysis patients. It is desirable to measure the serum aminotransferase levels at least once a month even in asymptomatic patients.

4. It is recommended to perform the HCV antibody test and, if necessary, the HCV-RNA test at the introduction of dialysis and the acceptance of patients. (Evidence level: Low, Recommendation level: Strong)

In HCV-positive chronic nephritis, there has been a report that membranoproliferative glomerulonephritis was the most frequent, accounting for 54%, that cryoglobulinemia was noted in 54% of the patients, and that HCV-RNA was detected in 66% on cryoprecipitation and 22% of frozen sections (17). Immunocomplexes are noted in the glomeruli by kidney biopsy, and they have been shown to be a cause of chronic nephritis such as membranoproliferative glomerulonephritis in which factors such as cryoglobulin are involved (18–21). The HCV antibody-positive rate is 7.9% in patients with kidney diseases compared with 1.03% in healthy individuals and is particularly high (16.6%) in patients with glomerulonephritis. This rate is higher in those patients with a Ccr level of less than 30 mL/min than in patients with a Ccr level of 30 mL/min or higher (13% vs. 2.7%). Furthermore, HCV infection has been reported to be involved in the etiology of glomerulonephritis (22). There has also been a report that HCV was positive in 3.9% of the 1041 CKD patients, and that 95% of HCV-positive patients showed viremia, and that the HCV-positive rate is high in CKD patients (23). It has also been reported that HCV antibody was positive in 12.7% of dialysis patients, and that of the dialysis patients, the HCV-antibody-positive rate was higher in those with non-insulin dependent diabetes mellitus (NIDDM) (20.8%) than in those with no diabetes mellitus (DM) (10%) (24), and that the HCV-positive rate in NIDDM patients was high at 19.5% (25). Based on these reports, HCV infection is likely to be involved in the pathogenesis of chronic kidney diseases. Therefore, the HCV-antibody-positive rate has been reported to be high at 7.3% (26) or 14.4% (27) in dialysis patients at the introduction of dialysis therapy. Moreover, according to the Dialysis Outcomes and Practice Patterns Study (DOPPS), the HCV-positive rate varied from 2.6% to 22.9% among the participating countries, and its increases were related to the dialysis period, male gender, black race, diabetes status, HBV infection, kidney transplantation, and alcohol and drug dependence. Many other studies have clarified the wide differences in the HCV-antibody-positive rate and the HCV-antibody-positive-conversion rate among dialysis patients at different facilities (28,29). Particularly, the HCV-positive-conversion rate has been reported to be high at facilities with a high HCV-positive rate

(30). Therefore, it is recommended to perform HCV antibody or HCV-RNA test at the introduction of dialysis therapy or at transfer of patients to another hospital.

5. In dialysis patients, it is desirable to perform the HCV antibody test at least once every 6 months even if HCV antibody is negative on the initial test. (Evidence level: Low, Recommendation level: Weak)

While there is no evidence concerning the frequency of HCV antibody test in dialysis patients, HCV positivity was reported to be detected in 70 days (36–210 days) by second-generation enzyme immunoassay (EIA) and in 49 days (27–119 days) by the third generation EIA from the detection of abnormality of ALT. In patients with acute HCV hepatitis, HCV-RNA becomes detectable in 1–2 weeks after HCV infection, and chronic HCV hepatitis is diagnosed when HCV-RNA persists for 6 months or longer. The chronicity rate is 55–85%. In acute HCV hepatitis cases, the ALT level begins to increase 2–8 weeks after infection. Symptoms usually appear 3–12 weeks (mean 7 weeks) after infection, and HCV antibody become positive simultaneously or with a slight delay. If the infection takes a chronic course, the ALT level increases and changes. Some immune-deficient individuals remain HCV-antibody-negative even after HCV infection (31). In a previous study, the HCV-RNA-positive-rate increased from 12.9% to 15.7% after a 4-year follow-up, de novo HCV infection was observed in one patient during this period with an HCV-positive-conversion rate of 0.33%/year, and the initial examination is considered to have been made during the window period in five of the patients, so that it was concluded that the HCV-RNA test must be performed once a month to reduce nosocomial HCV infection (32).

Also, there is a report that the HCV-antibody positive conversion rate was 0.44%/year when examined at 6-month intervals while observing the CDC standard preventive measures (33). Therefore, the KDIGO guidelines recommend to perform the HCV antibody test in HCV-antibody-negative patients once every 6–12 months (intermediate recommendation level) (34). The KDIGO also recommends testing by the enzyme antibody method at facilities with a low HCV infection rate and by the nucleic acid amplification technique at those with a high HCV infection rate (intermediate recommendation level) (34).

Based on these observations, it is considered desirable to perform the HCV antibody test at least once every 6 months in dialysis patients even if the HCV antibody were negative on the initial test.

6. If the serum aminotransferase level increases with no clear cause, it is recommended to perform an ad hoc HCV-RNA test or HCV core antigen test in addition to the HCV antibody test. (Evidence level: Low, Recommendation level: Strong)

If the serum aminotransferase level has increased with no obvious reason, there is the possibility of HCV infection. It has been reported that 9% of dialysis patients were HCV-RNA-positive even if they were HCV-antibody-negative, and the viral level is considered to have been low in such patients. Caution is needed in immune-deficient individuals such as dialysis patients because of a low viral level (35). Therefore, HCV infection cannot be excluded on the basis of a negative HCV antibody test, the HCV-RNA test must be performed when considered necessary. For the HCV-RNA assay, real-time PCR is recommended because of its high sensitivity (36,37). It has also been reported that patients become positive for the HCV core antigen 2 days after HCV infection but do not become positive for the HCV antibody until 50.8 days after infection. Thus a high-sensitivity assay for the HCV core antigen that is an inexpensive and quick method for the judgment of HCV infection, is useful for the diagnosis of HCV infection and is used during the window period until HCV antibody becomes positive (38,39). KDIGO recommends that the HCV test by a nucleic acid amplification technique should be carried out if the serum aminotransferase level has increased with no clear reason (strong recommendation) (34). Also, the determination of the viral level and HCV genotype by the HCV-RNA assay contributes to the evaluation of responses to interferon therapy (36). Thus if the serum aminotransferase level has increased with no clear cause, it is recommended to perform the HCV-RNA or HCV core antigen test ad hoc in addition to the HCV antibody test.

7. If an HCV-positive patient considered to be due to nosocomial infection has been detected, it is recommended to perform the HCV-RNA or HCV core antigen test in all dialysis patients who may have been exposed. (Evidence level: Very low, Recommendation level: Strong)

If a patient is judged to be newly positive on the HCV antibody test, the possibility of a nosocomial outbreak of HCV infection must be examined. As mentioned in the comment for Statement 6, the possibility of HCV infection cannot be excluded in patients who may have been exposed even if they are HCV-antibody-negative. Also, to fill the window period of HCV infection, a test for HCV-RNA or HCV core antigen must be performed. KDIGO rec-

ommends that surveillance to examine whether nosocomial infection has not occurred by the HCV-RNA test using a nucleic acid amplification technique be carried out if an HCV-positive patient considered to be due to nosocomial infection has been detected (strong recommendation). In addition, KDIGO recommends re-examination within 2–12 weeks after an initial negative examination (weak recommendation) (34).

Therefore, if an HCV-positive patient considered to be due to nosocomial infection has been detected, it is recommended to carry out the HCV-RNA or HCV core antigen test in all dialysis patients who may have been exposed.

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MANAGEMENT OF HEPATITIS C IN DIALYSIS PATIENTS (METHODS, FREQUENCY OF BLOOD TESTS AND IMAGING STUDIES)

[Statements]

1 Similar to patients with normal renal function, liver biopsy is the most reliable method to evaluate the liver disease of HCV-infected dialysis patients. It is mostly recommended, when transplantation is considered. (Evidence level: Low, Recommendation level: Weak)

2 The prognosis is significantly worse in HCV-infected dialysis patients than in uninfected dialysis patients. (Evidence level: High, Recommendation level: None)

3 It is recommended to periodically follow-up HCV-infected dialysis patients to screen for liver cirrhosis and early detection of hepatocellular carcinoma. (Evidence level: High, Recommendation level: Strong)

4 Iron has hepatocyte toxicity, and excessive hepatic iron deposition is an exacerbating factor of chronic hepatitis C and promotes hepatocarcinogenesis. In consideration of these facts, it is desirable to avoid iron overload in HCV-infected dialysis patients. (Evidence level: Low, Recommendation level: Weak)

[Comments]

1. Evaluation of the liver disease in HCV-infected dialysis patients

Similar to HCV-infected patients with normal renal function, liver biopsy is the most reliable method to evaluate the liver disease in HCV-infected dialysis patients. It is mostly recommended when kidney transplantation is considered. (Evidence level: Low, Recommendation level: Weak)

In dialysis patients, the aminotransferase levels are often low even when they are infected with HCV, and liver biopsy is the most reliable method to evaluate the liver disease of HCV-infected dialysis patients as well as HCV-infected patients with normal renal function.

Concerning histological changes of the liver, there have been many reports that inflammation and fibrosis are observed less frequently in HCV-infected dialysis patients than in HCV-infected patients with normal renal function (1–5). Cotler et al. (3) showed that HCV-infected dialysis patients had less inflammatory activity and a lower proportion of bridging fibrosis or cirrhosis than in hepatitis C patients with normal renal function. In addition, as a histological finding by liver biopsy, Shiavon et al. (4) and Hu et al. (6) reported that HCV-infected dialysis patients showed stage III and IV severe fibrosis significantly less frequently than those with normal renal function. Also, Sterling et al. (7) noted that the severity of liver fibrosis and liver cirrhosis was similar to that in hepatitis C patients with normal renal function showing a normal ALT level but was milder than in those showing a high ALT level. There is also a report that the progression rate of liver fibrosis corrected for the infected period was relatively slow (8). However, de Paula Farah et al. (9) have reported that histological findings of both fibrosis and inflammation are comparable between HCV-infected dialysis patients and HCV-infected patients with normal renal function.

On histological examination of the liver in HCV-infected dialysis patients before kidney transplantation, severe liver fibrosis or cirrhosis was noted in 5.5–32%, and liver cirrhosis was noted in 0–24% (2,3,5–8,10,11). The survival rate of dialysis patients with biopsy-proven cirrhosis during 10 years after transplantation was low at 26%, indicating that liver cirrhosis is an independent risk factor of poor prognosis, and liver cirrhosis is a contraindication for kidney transplantation (12). It has also been clarified that the prevalence of liver disorders after transplantation increases markedly (five times) if there is HCV infection before transplantation (13), and that the progression of hepatic lesions is faster in HCV-infected kidney transplantation patients than in HCV-infected patients with normal kidney function (14). Since the results of blood tests are not correlated with these histological changes of the liver, it is necessary to evaluate histological changes by liver biopsy before kidney transplantation (5,7,10,11,15,16).

In dialysis patients, it has been reported that percutaneous liver biopsy can be performed safely (17), but it generally increases the risk of hemorrhage. Transjugular liver biopsy is safer but is not performed widely.

2. Prognosis of HCV-infected patients

The prognosis is significantly worse in HCV-infected dialysis patients than in uninfected dialysis patients. (Evidence level: High, Recommendation level: None)

In 90% or more of dialysis patients, HCV infection leads to chronic hepatitis (18). The effects of HCV infection on the prognosis of dialysis patients have become an important issue due to the increase in patients with longer dialysis duration.

Many studies have indicated that the prognosis of HCV-infected dialysis patients is significantly worse than that of uninfected dialysis patients (19–25). According to meta-analysis by Fabrizi et al. (26), adjusted relative risk of all-cause mortality in HCV-infected dialysis patients was 1.34 on the basis of seven clinical studies involving 11 589 patients. Causes of death related to liver diseases such as hepatocellular carcinoma and liver cirrhosis were 5.89 times more frequent in the former group.

The incidence of liver cirrhosis in HCV-infected dialysis patients varies among reports from 1.3–12.5% (10,11,16,27). According to the investigation by Akiba et al. (28), the incidence of liver cirrhosis in HCV-antibody-positive dialysis patients was 8.57/1000/year.

There have been a few reports that the prognosis of liver disease is better in HCV-infected dialysis patients than in patients with normal renal function. Okuda et al. (29) reported that none of the 189 patients with HCV-infected dialysis patients showed progression to liver cirrhosis. Also, Ishida et al. (30) showed by a questionnaire survey of 6366 dialysis patients that hepatocellular carcinoma and liver cirrhosis were observed in 1.8% and 8.6%, respectively, which were lower than the percentages in patients with normal renal function. However, reports regarding the progression of liver diseases have been inconsistent, with an 8-year prospective cohort study by Espinosa et al. (31) showing the rapid progression to liver cirrhosis in dialysis patients, being observed after a median of 7 years from the initial elevation in ALT, which is in contrast to the general population.

Generally, the incidence of hepatocellular carcinoma in HCV-infected patients is proportionate to the severity of liver fibrosis, and its incidence in patients with liver cirrhosis showing severest fibrosis is reported to be about 8%/year (32). However, there is no detailed report on the incidence of hepatocellular carcinoma in HCV-infected dialysis patients. Nakayama et al. (20) followed up 276 HCV-antibody-positive dialysis patients over 6 years and reported liver cirrhosis in 30 and hepatocellular carcinoma in eight at the end of the follow-up period. If most hepatocellular carcinomas are assumed to have occurred in liver cirrhosis, the annual rate of progression from liver cirrhosis to hepatocellular carcinoma is considered to be at least 4%. The finding that liver cirrhosis was noted in 30 (13.2%) of the 276 patients suggests that the progression rate to liver cirrhosis is nearly the same as that in non-dialysis patients.

In dialysis patients, the incidence of, and mortality due to, cancers have often been reported to be higher than in the general population. According to a report from Italy, the incidence of hepatocellular carcinoma is 2.41 times higher in dialysis patients than in those with normal renal function (33). According to a study in Okinawa, Japan, the incidence of cancer in dialysis patients was 2.48 times higher in males and 3.99 times higher in females than that in the general population, but the incidence of hepatocellular carcinoma was similar in males and lower in females compared with that in the general population (34). In a prospective study of a cohort of 233 HCV-infected dialysis patients, hepatocellular carcinoma was observed in three patients during 10 years (0.53%/year) (35). According to a questionnaire survey of 67 970 patients, the incidence of hepatocellular carcinoma was reported to be 3.87/1000 HCV-infected dialysis patients/year during a 3-year period (28).

At the end of 1999, the prevalence of liver cirrhosis was 8.25% and 11.84% in HCV-antibody-positive patients and HCV-RNA-positive dialysis patients, respectively, and that of hepatocellular carcinoma was 2.16% and 2.59%, respectively. In those coinfected with HBV and HCV, the prevalences of liver cirrhosis and hepatocellular carcinoma were 12.2% and 2.7%, respectively (36). In patients coinfected with HBV and HCV, liver damage is notable even in those with normal renal function. However, as the same is observed also in HCV-infected dialysis patients (8), particularly close follow-up is needed.

To date, there has been no control study comparing the prognosis between HCV-infected dialysis patients and HCV-infected patients with normal renal function. This comparison may be difficult because of the reduced life expectancy in dialysis patients.

There has been no report on the prognosis-improving effect of therapeutic intervention in HCV-infected dialysis patients.

Reports on the viral load level in dialysis patients have been inconsistent: It has been reported to be low by some (37,38), not to differ by others (2,39), and to be high in still others (6). The HCV RNA levels were reported to decrease in dialysis patients but not to change in the control group during a 3-year follow-up by Furusyo et al. (38) and during a 10-year follow-up by Okuda et al. (29), respectively.

In a comparison concerning comobidities, hypertension, hepatitis B, liver cirrhosis, wasting, anemia, and HIV infection were more prevalent, but coronary artery disease and stroke were less prevalent in 5737 HCV-infected dialysis patients than in 11 228

uninfected dialysis patients matched for the time at which dialysis was initiated. On the other hand, there is also a report that coronary artery disease was more prevalent in HCV-infected dialysis patients (40).

3. Follow-up

It is recommended to periodically follow-up HCV-infected dialysis patients for the diagnosis of liver cirrhosis and early detection of hepatocellular carcinoma. (Evidence level: High, Recommendation level: Strong)

HCV-infected dialysis patients develop liver cirrhosis or hepatocellular carcinoma more frequently than uninfected dialysis patients, and periodic follow-up for the diagnosis of liver cirrhosis and early detection of hepatocellular carcinoma is necessary.

Follow up testing to evaluate the progression of liver disease (liver fibrosis, liver cirrhosis, hepatocellular carcinoma) include blood tests of AST, ALT, γ-GTP, total bilirubin, albumin, platelet count, and AST/platelet ratio and imaging techniques such as abdominal ultrasonography and contrast-enhanced CT.

Since the AST and ALT levels are low in dialysis patients regardless of the presence or absence of liver disease, blood tests of liver fibrosis are necessary as well as those of AST and ALT for the follow-up of dialysis patients. In patients with chronic hepatitis C, in general, the platelet count has been reported to reflect liver fibrosis (41). The platelet count is also useful as a marker of liver fibrosis in dialysis patients (4). In HCV-infected dialysis patients, it has been reported that platelets decrease with time compared with uninfected dialysis patients and that the increases in ALT and decreases in the platelet count are related (42).

Generally, a high AST level as well as a low platelet count is related to liver fibrosis, and the AST (IU/L)/platelet count ($\times 10^4/\mu L$) ratio is useful as a marker of liver fibrosis. This marker is also useful in dialysis patients, indicating no fibrosis when it is less than 0.40 but fibrosis when it is 0.95 or higher (4,7).

In dialysis patients with liver cirrhosis, a high ALT level and low albumin, total cholesterol, and white blood cell count have been reported in addition to a low platelet count (36).

Ultrasonography is also considered useful for the dialysis of liver disorders in dialysis patients, and ultrasound findings are correlated with the hyaluronic acid level and platelet count (35).

The concentrations of α-fetoprotein and PIVKA-II, which are markers of hepatocellular carcinoma, can be interpreted in dialysis patients similar to patients with normal renal function (43,44).

Since some dialysis patients as well as patients with normal renal function are positive for HCV antibody but negative for HCV-RNA, the HCV-RNA test is necessary if HCV antibody is positive.

There is no evidence concerning the frequency of follow-up tests.

In Japan, there was a nationwide survey of the state of execution of tests for viral hepatitis in dialysis patients, particularly those for the detection of hepatocellular carcinoma, in 2009 (45). According to this survey, periodic follow-up using imaging techniques including ultrasonography and CT are performed in patients positive for hepatitis virus at 80% of the facilities, and the frequency of the follow-up was less than once a year in 5.4%, once a year in 56.5%, two times a year in 28.8%, and three or more times a year in 9.3%. Tumor markers were measured periodically at only 48.9% of the facilities, and the establishment of follow-up plans and systems according to the guidelines is anticipated.

The KDIGO guidelines recommend that follow up testing for HCV-related comobidities (such as liver cirrhosis and hepatocellular carcinoma) should be performed every 6 months in patients with liver cirrhosis and every year in those without liver cirrhosis (46).

However, the working group proposes the more close follow-up plan for the detection of hepatocellular carcinoma on the basis of the follow-up plan for patients with chronic hepatitis C recommended by the Japan Society of Hepatology (47).

Patients with chronic hepatitis, patients with a platelet count of $10^5/\mu L$ or higher
Tests: AFP, PIVKA-II, abdominal ultrasonography (about once every 6 months-1 year)
Liver cirrhosis patients, patients with a platelet count of less than $10^5/\mu L$
Tests: AFP, PIVKA-II, abdominal US (about once every 3 months), contrast-enhanced CT (about once every 6 months)

If contrast-enhanced CT cannot be performed, or if the diagnosis is difficult, MRI using EOB containing a small amount of gadolinium, which, in principle, should be substituted for another test in dialysis patients, should be considered.

A test of the AFP-L3 fraction must be considered when the AFP level is high.

4. Administration of iron preparations

Iron has hepatocyte toxicity, and excessive hepatic iron deposition is an exacerbating factor of chronic hepatitis C and promotes hepatocarcinogenesis. In consideration of these facts, it is desirable to avoid

iron overload in HCV-infected dialysis patients. (Evidence level: Low, Recommendation level: Weak)

Iron is a trace element indispensable for hemoglobin synthesis. Iron stored in the liver is released into blood when necessary. It has been shown that iron has hepatocyte toxicity and that excessive hepatic iron deposition is an exacerbating factor of chronic hepatitis C and promotes hepatocarcinogenesis. Patients with chronic hepatitis C show excessive iron deposition in liver tissue, and iron-dependent oxidative stress has been suggested to be involved in various stages including hepatocyte damage, fatty degeneration, fibrosis, and carcinogenesis. Iron deposition in the liver has also been reported to be related to hyporesponsiveness to interferon therapy in patients with normal renal function (48). Moreover, iron depletion therapy has been reported to significantly lower the risk of hepatocellular carcinoma in hepatitis C patients (49).

In hemodialysis patients, also, the serum ferritin level shows significant positive correlations with the AST and ALT levels in those positive for HCV antibody (50). There has been no large-scale clinical study evaluating the effects of iron administration on the liver in HCV-infected dialysis patients. Kurihara et al. (51) administered an intravenous iron preparation to HCV-antibody-positive dialysis patients for one year with a target serum ferritin level of 200–300 ng/mL, though the number of patients was small, observed changes in the liver function, and compared them with those in HCV-antibody-negative dialysis patients. According to this study, the AST and ALT levels increased in two of the seven HCV-antibody-positive patients but could be controlled by the administration of stronger neo-minophagen C, no change was observed in other markers such as the viral level, cholinesterase level, and platelet count, and the administration of an iron preparation to HCV-antibody-positive patients was safe. In their study, however, no histological evaluation was made, and long-term consequences are unknown. Kato et al. (52) showed that the oxidative stress marker levels were high in HCV-infected dialysis patients and were increased further by the administration of an iron preparation.

On the other hand, HCV-infected dialysis patients have been shown to have a high endogenous erythropoietin concentration and need a lower dose of erythropoietin (53). This is considered to be due to an increase in the erythropoietin production by hepatocytes in the process of hepatocyte regeneration. The same report showed that they also require a lower dose of iron. This is considered to be due to the release of iron stored in hepatocytes induced by inflammation, causing an increase in ferritin.

From these observations, caution to avoid iron overload is necessary in administering iron preparations to HCV-infected dialysis patients. Therefore, in HCV-infected dialysis patients, iron supplementations should be restricted to anemia not responding even to the maximum dose of an ESA preparation (54).

Package inserts mention severe liver disorder as a contraindication of intravenous iron preparations.

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INDICATIONS OF ANTIVIRAL THERAPIES IN DIALYSIS PATIENTS

[Statements]

1. Performance of antiviral therapy in HCV-infected dialysis patients is recommended if the prognosis is expected to be improved. (Evidence level: Very low, Recommendation level: Strong)
2. Performance of antiviral therapy in HCV-infected dialysis patients is recommended in case of expecting kidney transplantation. (Evidence level: High, Recommendation level: Strong)
3. If a dialysis patient has contracted acute HCV infection and the virus cannot be eliminated within 12 weeks spontaneously, performance of antiviral therapy is desirable. (Evidence level: High, Recommendation level: None)

[Comments]

1. Performance of antiviral therapy in HCV-infected dialysis patients is recommended if the prognosis is expected to be improved. (Evidence level: Very low, Recommendation level: Strong)

Dialysis patients are at high-risk of HCV infection, and many patients are suffering from chronic hepatitis C. Patients with chronic hepatitis C tend to develop liver cirrhosis and hepatocellular carcinoma during its long-term course (1,2). While HCV infection has been reported to increase the mortality due to liver cirrhosis and/or hepatocellular carcinoma in dialysis patients, prognosis of HCV-infected dialysis patients is known to be poor regardless of the presence or absence of liver disease (3–5). In Japan, patients who are undergoing dialysis for 20 years or longer are not rare (6), and thus the management of HCV infection, which affects the prognosis, is important.

HCV can be eliminated by antiviral therapy using interferon (IFN), and viral elimination contributes to the control of hepatitis and prevention of its progression to liver cirrhosis or hepatocellular carcinoma. In the past, introduction of antiviral therapy tended to be uncertain in dialysis patients with HCV infection, while recently, we came to consider that antiviral therapy should be performed aggressively in dialysis patients in whom long-time survival is expected. According to a survey by the Japanese Society for Dialysis Therapy, 48% of the anti-HCV-positive dialysis patients are HCV RNA-positive (7), and many of these HCV RNA-positive patients are considered to have indications of antiviral therapy. Antiviral therapy not only improves the prognosis of the HCV-infected patients themselves but also reduces sources of infection to other patients. Presently, most new HCV infections in dialysis patients are considered to be nosocomial ones (8). Thus, antiviral therapy should further be considered in HCV-infected patients.

A basic consensus has been made concerning the indications of antiviral therapy for chronic hepatitis C in patients with normal renal function (9,10). Guidelines for antiviral therapy for chronic hepatitis C patients with reduced renal function, which must be evaluated individually, have not been issued for a long time. Recently, guidelines for the treatment of hepatitis C in patients with chronic kidney disease (11) have been proposed by KDIGO (Kidney Disease: Improving Global Outcomes), and patients whose prognosis is expected to be improved are considered to have indications for aggressive antiviral therapy. The KDIGO Guideline defines patients whose prognosis is expected to be improved as young patients who have no severe cardiovascular complication and are expected to live for at least 5 years. The Japanese guideline is created along with this proposal.

In selecting patients with indications for antiviral therapy, the severity of liver disorder, age, comobidities, and tolerability to treatment are important factors, and candidates are selected in consideration of the therapeutic effect and the patient's condition (12,13). Particularly, patients in whom IFN is expected to be effective from the viewpoint of cost-effectiveness are optimal candidates for aggressive treatment. Among the predictive factors of the effectiveness of IFN accumulated in non-dialysis patients, those that predict marked response to IFN, i.e. SVR (sustained virological response) are: (i) As factors of HCV, (1) a low viral load and (2) HCV genotypes other than 1a and 1b; (ii) as host factors, (1) no advanced fibrosis (≤F3 according to the New Inuyama Classification), (2) age under 45 years, (3) a 5-year or shorter infection period, (4) no obesity, and (5) a low γGTP level (14,15). According to data in Japan, IFN therapy is expected to suppress hepatocarcinogenesis even if SVR cannot be achieved (15). Incidentally, liver biopsy is reliable for the evaluation of liver fibrosis, but liver fibrosis can also be estimated to an extent from the platelet count, liver fibrosis markers, AST/platelet count ratio, and findings on abdominal ultrasonography (16).

The present consensus is that there is no age restriction for administering antiviral therapy, but as

the response rate to IFN is low, and the frequency of the occurrence of adverse effects is high, in patients aged 65 years or older, whether they should be treated aggressively needs careful evaluation in considering their prognosis. Also, severe complications, e.g., psychiatric disorders such as depression, severe hypertension, heart failure, significant coronary artery disease, poorly controlled diabetes, chronic obstructive pulmonary disease, untreated thyroid disease, uncompensated liver cirrhosis, and active or suspected malignancy, are contraindications for the treatment (12,13). Patients with poor compliance and children are also excluded. In antiviral therapy for patients with normal renal function, peginterferon (PEG-IFN) and ribavirin are usually used in combination. However, ribavirin is contraindicated, in principle, because it causes hemolytic anemia that can be particularly dangerous in dialysis patients and cannot be eliminated by dialysis, so the treatment using PEG-IFN alone is generally recommended. The SVR rate achieved by PEG-IFN in dialysis patients is similar to or better than that in non-dialysis patients, but the frequency of adverse effects and dropout rate of the therapy are slightly higher (17–19).

Recently, antiviral therapy has become recommended in HCV carriers with normal renal function showing persistently normal ALT (PNALT) (20), because it has been learned that the risk of progression of liver fibrosis (i.e. hepatocarcinogenesis) is high in many patients with a platelet count of 150 000/mm^3 or below regardless of the ALT level (21). In Japan, a treatment guideline setting an ALT of 30 IU/mL and a platelet count of 150 000/μL as cut-off values (22) for PNALT patients has already been prepared. The ALT level is significantly lower in dialysis patients than in patients with normal renal function, and patients with a low ALT level may have liver disorders. Therefore, antiviral therapy should be considered regardless of the ALT level.

2. Performance of antiviral therapy in HCV-infected dialysis patients is recommended in case of expecting kidney transplantation. (Evidence level: High, Recommendation level: Strong)

Many patients waiting for kidney transplantation are young, have few serious complications, and are expected to survive over a long period. Further, the prognosis is expected to be more favorable in patients after successful kidney transplantation than in dialysis patients. Therefore, antiviral therapy is positively recommended to patients waiting for kidney transplantation.

In HCV-antibody-positive recipients of kidney transplantation, both the survival rate and graft survival rate are reported to be lower than in HCV-antibody-negative recipients (23,24). In principle, antiviral therapy is not recommended after kidney transplantation, because it may induce rejection or exacerbate liver disorders. However, elimination of HCV by antiviral therapy from patients waiting for kidney transplantation is expected to not only prevent the exacerbation of hepatitis after transplantation, avoid graft loss by preventing hepatitis C-related nephropathy and acute rejection, and suppress the occurrence of new diabetes but also improve the prognosis (25,26).

3. If a dialysis patient has contracted acute HCV infection and the virus cannot be eliminated within 12 weeks spontaneously, performance of antiviral therapy is desirable. (Evidence level: High, Recommendation level: None)

The therapeutic effect of IFN in patients with acute hepatitis C is higher than in those with chronic hepatitis C. IFN therapy is particularly effective if conducted early after the onset of acute hepatitis, and as high as over 80% of SVR rate can be expected (27,28). However, acute hepatitis C cures spontaneously in some patients within 12 weeks after the onset (29), and the possibility of spontaneous HCV RNA clearance in the general population has been reported to be 30–50% (29,30). However, 12 or more weeks after the onset, the disease rarely cures spontaneously and often takes a chronic course. Therefore, IFN therapy is recommended to be initiated as early as possible in patients not showing seroclearance of HCV-RNA within 12 weeks after the onset. Early initiation of treatment is particularly necessary in genotype 1 (28). If the treatment is initiated after 20 weeks, the condition approaches chronic hepatitis, and the SVR rate declines (28). The SVR rate improves with the duration of IFN therapy, and the administration should be continued for 24 weeks in patients with genotype 1 and for 8–12 weeks in those with other genotypes (31). The incidence of acute hepatitis C is high in dialysis patients, and its spontaneous cure rate is 5–30% (1), which is lower than in the general population. Therefore, IFN therapy for acute hepatitis C should be conducted more actively in patients on dialysis than in those not on it. There have been some reports indicating that SVR rate tends to be lower in dialysis patients than in patients with normal renal function (32,33). However, it is generally considered that antiviral therapy such as IFN therapy is useful for the treatment of acute hepatitis C even in patients on hemodialysis.

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TREATMENT OF DIALYSIS PATIENTS BY ANTIVIRAL THERAPIES

[Statements]

1 It is recommended that for dialysis patients with HCV infection, interferon of antiviral therapy is the first choice.

2 In dialysis patients, the response rate of interferon therapy is comparable or superior to that in patients with normal renal function, but as the frequency of adverse effects is also high, sufficient observation is recommended. (Evidence level: Low, Recommendation level: Strong)

3 Since the blood levels of both standard interferon α and pegylated interferon α increase excessively in dialysis patients if they are administered at standard dose, an adjusted dose reduction to the level of renal function is recommended. (Evidence level: High, Recommendation level: Strong)

4 It is recommended not to use ribavirin contraindicated in dialysis patients. (Evidence level: High, Recommendation level: Strong)
5 The therapeutic guidelines for patients with normal renal function mention the selection of drugs depending on the viral level and viral type and whether ribavirin should be used concomitantly. However, there is no recommendation for the selection of drugs according to the viral level or viral type for dialysis patients, in whom ribavirin administration is a contraindication.
6 In dialysis patients, treatment with pegylated interferon α monotherapy is more effective and less frequently causes adverse effects than treatment with standard interferon α monotherapy. (Evidence level: High, Recommendation level: Strong)
7 Interferon β can be used in dialysis patients at the standard dose
   • However, as its intravenous injection over a short period may cause adverse effects due to a rapid increase in its plasma concentration, it is recommended to administer it by intravenous drip infusion over 30–60 min for dialysis patients. (Evidence level: High, Recommendation level: Strong)
8 It is recommended that HCV-infected dialysis patients accepted for kidney transplantation be treated before transplantation. (Evidence level: High, Recommendation level: Strong)
9 It is recommended that treatment of HCV-infected kidney transplant recipients be considered only when the benefits of treatment clearly outweigh the risk of allograft rejection due to interferon therapy. (Evidence level: High, Recommendation level: Strong)

[Comments on treatments for HCV-infected dialysis patients]

1. Treatment with interferon (IFN) monotherapy

Monotherapy with standard interferon. Many of the studies of IFN therapy for dialysis patients have been case reports of a small number of patients, making its evaluation difficult. According to the reports of a relatively large number of patients published since 2000, the sustained virological response (SVR) rate varies widely from 19% to 62% (1–5).

The results of meta-analyses of treatments using IFNα monotherapy including these reports are reviewed. In the reports by Fabrizi et al., which covered 28 studies and 645 dialysis patients, the SVR rate by treatment using IFNα monotherapy was 39%, and the dropout rate from the treatment was 19% (6). According to the report by Gordon et al., which reviewed 20 studies and 459 dialysis patients, the SVR rate by IFNα monotherapy was 41%, and the dropout rate was 26% (7). Important factors that contributed to SVR were the administration of IFNα at 3 MU or above 3 or more times/week, a low HCV-RNA level, mild liver fibrosis, and a genotype other than genotype 1. In all meta-analyses, the effectiveness of IFN was similar or superior, but the dropout rate due to adverse effects was higher, in dialysis patients compared with patients with normal renal function. Since the treatment is discontinued more frequently due to cytopenia and psychiatric symptoms in dialysis patients than in patients with normal renal function, sufficient observation and management are needed.

Also, concerning the pharmacokinetics of IFNα2b, the AUC and Cmax are about two times higher, and the half-life is also prolonged in dialysis patients compared with patients with normal renal function. In dialysis patients, the dose must be reduced to a half of the usual dose for patients with normal renal function or below (8).

Monotherapy with IFNβ. As for studies on treatments using IFNβ monotherapy, there are data of 20 patients reported by Zeniya et al. in Japan. These patients, consisting of 60% genotype 1 (12/20) and 40% genotype 2 (8/20), in whom the HCV-RNA level was 15–150 KIU/mL, showed a high SVR rate of 90% (18/20) with no serious adverse effect such as depression during administration (9). There has been no other report of a large number of dialysis patients who underwent IFNβ therapy, and the SVR rate in dialysis patients is unclear. In Japan, however, IFNβ has been used widely in patients with normal renal function, and both its efficacy and safety are established.

Concerning the pharmacokinetics of IFNβ, the peak plasma concentration is higher in dialysis patients than in patients with normal renal function, but its half-life in dialysis patients does not differ markedly compared with that in patients with normal renal function, and there is no tendency for accumulation. Therefore, it is considered possible to administer IFNβ to dialysis patients at the same dose as in patients with normal renal function. Except, in dialysis patients, its intravenous injection over a short period has been reported to induce adverse effects such as headache, nausea, and a decrease in the blood pressure due to a rapid increase in its plasma concentration. Therefore, in dialysis patients, it is recommended to conduct IFNβ therapy by intravenous drip infusion over about 30–60 min (10–14).