

Protective effects of hydrogen gas inhalation on radiation-induced bone marrow damage in cancer patients: a retrospective observational study

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Abstract

Although intensity-modulated radiation therapy (IMRT) has been developed as an alternative to conventional radiotherapy, reducing bone marrow damage is limited. Thus, a novel technology is needed to further mitigate IMRT-induced bone marrow damage. Molecular hydrogen (H₂) was recently reported as a preventive and therapeutic antioxidant that selectively scavenges hydroxyl radical (\cdot OH) and peroxynitrite (ONOO⁻). This observational study aimed to examine whether H₂ gas treatment improves IMRT-induced bone marrow damage in cancer patients. The study was performed at Clinic C4 in Tokyo, Japan between May 2015 and November 2016. During this period, all enrolled patients received IMRT once per day for 1 to 4 weeks. After each time of IMRT, the patients of control group ($n = 7$, 3 men and 4 women, age range: 26–70 years) received mild hyperbaric oxygen therapy in health care chamber for 30 minutes, and the patients of H₂ group ($n = 16$, 8 men and 8 women, age range: 35–82 years) received 5% H₂ gas in health care chamber for 30 minutes once per day. Radiation-induced bone marrow damage was evaluated by hematological examination of peripheral blood obtained before and after IMRT, and the data were expressed by the ratio after to before treatment. The total number of radiation times and total exposure doses of radiation were similar between the control and H₂ groups. IMRT with health care chamber therapy significantly reduced white blood cells and platelets, but not red blood cells, hemoglobin and hematocrit. In contrast, H₂ gas treatment significantly alleviates the reducing effects of white blood cells and platelets ($P = 0.0011$ and $P = 0.0275$, respectively). Tumor responses to IMRT were similar between the two groups. The results obtained demonstrated that H₂ gas inhalation therapy alleviated IMRT-induced bone marrow damage without compromising the anti-tumor effects of IMRT. The present study suggests that this novel approach of H₂ gas inhalation therapy may be applicable to IMRT-induced bone marrow damage in cancer patients. The study protocol was approved by an Ethics Committee Review of Tokyo Clinic and Research Institute ICVS Incorporated (Tokyo, Japan) on February 1, 2019, and was registered in the University Hospital Medical Information Network (UMIN) Clinical Trials Registry (UMIN ID: UMIN000035864) on February 20, 2019.

Key words: bone marrow damage; cancer patient; hydrogen gas; IMRT; intensity-modulated radiation therapy; platelet; radiation-induced damage; retrospective observational study; white blood cell

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INTRODUCTION

When the shape of a tumor is irregular and complex, conventional radiotherapy is unable to maintain a high tumor control rate and simultaneously attenuate the associated complications, because normal tissues and organs surrounding the tumor receive the same dose of irradiation. As an alternative to conventional radiotherapy, intensity-modulated radiation therapy (IMRT) has been developed to only irradiate tumors.¹ However, bone marrow damage still occurs in cancer patients with multiple tumor lesions and large irradiation volumes. Thus, novel technology is needed to mitigate further IMRT-induced bone marrow damage. To attenuate the symptoms associated with end-stage cancer, patients are also housed in a health care chamber (HCC, a mild hyperbaric oxygen chamber)²; however, satisfactory outcomes have not yet been achieved.

The direct effects of radiation are due to the changes it induces in bioactive macromolecules such as proteins

and nucleic acids. Ionization, excitation, chemical bond rupture, and changes in molecular structures occur during this process, leading to abnormal functions and metabolic disorders.³ Indirect effects also occur via the generation of free radicals by water radiolysis. Hydroxyl radicals (\cdot OH), the strongest reactive oxidant species, are formed from this radiolysis and react with nucleic acids, lipids, and proteins. Approximately 65% of DNA damage is caused by the indirect effects of free radicals such as \cdot OH.⁴ Therefore, selective and high concentrations of \cdot OH scavengers have potential as radioprotective agents.

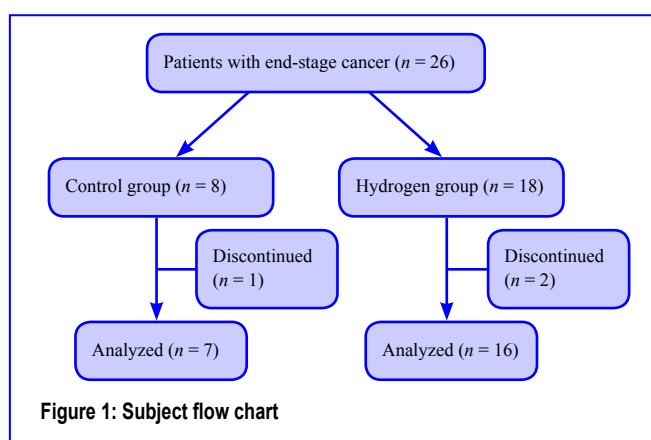
Molecular hydrogen (H₂) was recently identified by Ohsawa et al.⁵ as a preventive and therapeutic antioxidant that selectively scavenges \cdot OH and peroxynitrite (ONOO⁻). However, in 2005, 2 years before Ohsawa's study,⁵ Yanagihara et al.⁶ reported that the consumption of neutral H₂-rich water produced by electrolysis may effectively reduce the oxidative stress induced by chemical oxidants in rats, which is pioneering

research in H₂ medicine. Due to its small size and electrically neutral properties, H₂ easily reaches target organs. H₂ has also been proposed as a treatment for various oxidative stress-related diseases.⁷⁻⁹ Previous studies demonstrated that H₂ exerted radioprotective effects in various animal models^{10,11} and improved the quality of life (QOL) of patients treated with radiotherapy for liver tumors.¹² However, there is currently no definitive therapy to attenuate radiation-induced bone marrow damage in cancer patients. Therefore, this retrospective observational study was designed to investigate whether H₂ gas treatment mitigates IMRT-induced bone marrow damage in cancer patients.

SUBJECTS AND METHODS

Subjects and designs

The study was a retrospective observational examination performed at Clinic C4 in Tokyo, Japan between May 2015 and November 2016. During this period, 26 patients with end-stage cancer receiving two different treatments, IMRT with or without H₂ gas inhalation (the H₂ group and control group, respectively), were registered. Patients who did not receive all planned IMRT were excluded. One patient in the control group and two patients in the H₂ group did not receive all of the planned IMRT. Therefore, the data were collected from 23 patients, and the number of patients in the control group and H₂ group consisted of 7 and 16 patients, respectively.



The study protocol and materials were approved by an Ethics Committee Review of Tokyo Clinic and Research Institute ICVS Incorporated (Tokyo, Japan) on February 1, 2019 (**Additional file 1**), and all patients provided written informed consent (**Additional file 2**) prior to receiving therapy. All methods were performed in accordance with the relevant guidelines and regulations. The present study was registered in the University Hospital Medical Information Network (UMIN) Clinical Trials Registry (UMIN ID: UMIN000035864) on February 20, 2019.

Patients were subjected to 5 to 20 minutes of Tomo Therapy (HI-ART, Japan Accuray Inc., Tokyo, Japan) once per day for 1 to 4 weeks, except Saturday and Sunday. After each time of IMRT, the control group ($n = 7$, 3 men and 4 women) were housed in HCC (APF2, Air Press Co., Ltd., Tokyo, Japan) for 30 minutes under the environmental conditions of 1.35 atm and 27% O₂. The H₂ group ($n = 16$, 8 men and 8 women) were

also housed in HCC and received 5% H₂ gas inhalation for 30 minutes. H₂ gas was prepared by mixing H₂ gas and air using a 3.5% H₂ gas inhaler (MHG-2000, MiZ Co., Ltd., Kanagawa, Japan) and 6.5% H₂ gas inhaler (MHG-2000α, MiZ Co., Ltd.) connected in parallel. H₂ gas in both groups was produced by the electrolysis of water, and concentrations were controlled under the detonation limit of the mixture of H₂ gas and air.¹³ Patients received mixed gas via a nose cannula at a flow rate of 4 L/minute in HCC. All patients in this study did not receive chemotherapy or other standard therapies other than IMRT.

Evaluation of bone marrow damage

Bone marrow damage was evaluated by a hematological examination of peripheral blood. Blood samples drawn from the median cutaneous vein were obtained from all patients on the first day of IMRT (before the treatment) and after 1 to 4 weeks of IMRT (after the treatment). A blood hematology test for the counts of red blood cells (RBC), white blood cells (WBC), and platelets (PLT), the concentration of hemoglobin (HGB), and the hematocrit (HT) was conducted before and after the treatment using standard assays in a contract clinical examination laboratory, because these hematological markers can rapidly and easily assess the bone marrow damage in the daily work of hospital.

Evaluation of tumor responses

Patients underwent dynamic CT scans 1 month after completion of IMRT using iodine-based angiographic contrast agent, and then tumor response was checked at 2 to 3 months intervals. The tumor response was determined by the criteria established by Kwon et al.¹⁴ using the dynamic CT scans. Described briefly, complete response was defined as the disappearance of any intratumoral arterial enhancement in all target lesions. Partial response was defined as at least a 30% decrease in the sum of the diameters of viable target lesions. Progressive disease was defined as at least a 20% increase in the sum of the diameters of viable target lesions or the appearance of a new lesion. Stable disease was defined as a tumor status that did not meet any of the above criteria.

Statistical analysis

Hematological data were expressed as ratios of after to before the treatment. The data including the number of radiation times, total exposure dose of radiation calculated by multiplying the tumor volume of an individual patient by the radiation ($\times 10^3$ cm³·Gy), and hematological examinations were presented as means \pm standard errors of the means (SEM) and analyzed statistically by an unpaired Student's *t*-test or the Mann-Whitney *U* test where appropriate using BellCurve for Excel (Version 3.0, Social Survey Research Information Co., Ltd., Tokyo, Japan). $P < 0.05$ was considered to be significant.

RESULTS

Patient characteristics

Patient characteristics, including sex, age, tumor origin, stage of cancer, and number of metastases, are shown in **Table 1** and **Additional Table 1**, and were not significantly different between the control and H₂ groups.



Table 1: Baseline data of patients from control and H₂ groups

	Control	H ₂
<i>n</i>	7	16
Sex (male/female)	3/4	8/8
Age (yr)	54.6±8.3	65.9±3.5
Diagnosis		
Origin		
Ovary	1	2
Stomach	1	1
Colon	1	0
Lung	1	0
Prostate	2	3
Breast	1	1
Ureter	0	1
Liver	0	2
Bile duct	0	1
Pancreas	0	3
Urinary bladder	0	2
Metastasis	7/7	16/16
Stage		
IV	7	16

Note: Data are expressed as number, except age (mean ± SEM). No significant differences were observed in age between the control and H₂ groups by statistical analysis with unpaired Student's *t*-test. The stage of the patients was confirmed according to the TNM classification in Union for International Cancer Control.

Radiation conditions

As shown in **Figure 2A** the number of radiation times for the control and H₂ groups was 10.7 ± 1.3 and 11.2 ± 1.1 , respectively, which was not significantly different between the two groups ($P = 0.8064$). In addition, as shown in **Figure 2B**, the total exposure doses of radiation calculated by multiplying the tumor volume of an individual patient by the radiation were $446.0 \pm 101.8 \times 10^3 \text{ cm}^3 \cdot \text{Gy}$ and $410.2 \pm 124.4 \times 10^3 \text{ cm}^3 \cdot \text{Gy}$, respectively, which were also not significantly different between the two groups ($P = 0.8607$).

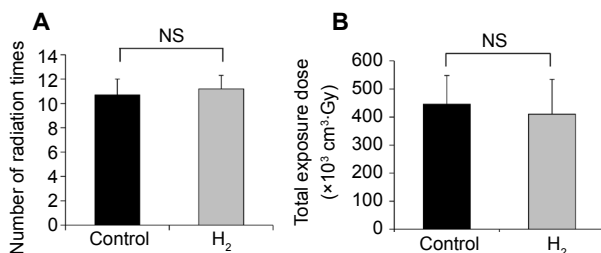


Figure 2: Radiation conditions of patients in control and H₂ groups

Note: (A) Number of radiation times for the control group and hydrogen (H₂) group. (B) Total exposure doses of radiation for these groups. Data are given as the means ± SEM ($n = 7$ in control group, and $n = 16$ in H₂ group), and were analyzed by Mann-Whitney *U* test. NS: Not significant.

Tumor responses

Tumor responses to IMRT were similar between the two groups, and 4 of 7 (57%) patients in the control group and 7 of 16 (44%) patients in the H₂ group achieved a complete or partial response. In addition, 3 of 16 (19%) patients in the H₂ group achieved stable disease. These results indicated that tumor responses to IMRT were similar between the two groups,

and that H₂ gas inhalation did not compromise the anti-tumor effects of IMRT. Moreover, the QOL, such as fatigue, sleep, and gastrointestinal symptoms, assessed based on the results of medical interviews was similar between the two groups, indicating that H₂ gas inhalation did not compromise the QOL of patients receiving IMRT.

Bone marrow damage

In the control and H₂ treatment groups, RBC ratios were 1.108 ± 0.124 and 0.985 ± 0.027 , HGB ratios were 1.078 ± 0.126 and 0.966 ± 0.029 , and HT ratios were 1.073 ± 0.121 and 0.953 ± 0.027 , respectively. These values were close to 1, and no significant differences were observed between the two groups (**Figure 3**), indicating that IMRT did not influence these hematological markers. In contrast, WBC ratios were 0.145 ± 0.041 and 0.612 ± 0.088 for the control and H₂ groups, respectively, and were significantly different ($P = 0.0011$; **Figure 4A**). PLT ratios were 0.337 ± 0.106 and 0.752 ± 0.130 for control and H₂ groups, respectively, and were also significantly different ($P = 0.0275$; **Figure 4B**). These results indicated that IMRT selectively reduced hematological markers, such as WBC and PLT, in the control group, whereas the H₂ gas treatment protected against these reductions.

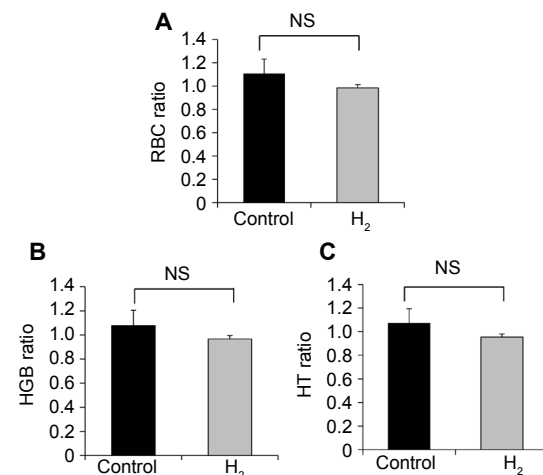


Figure 3: Effects of the hyperbaric hydrogen (H₂) gas treatment on the red blood cell counts (RBC, A), hemoglobin levels (HGB, B), and the hematocrit (HT, C) of patients in control and H₂ groups

Note: Blood samples were obtained from all patients on the first day of intensity-modulated radiation therapy (IMRT) (before treatment) and after 1 to 4 weeks of IMRT (after treatment). (A–C) RBC (A), HGB (B), and HT (C) were expressed as ratios of after to before the treatment. NS: not significant. Data are given as the means ± SEM ($n = 7$ in control group, and $n = 16$ in H₂ group), and were analyzed by Mann-Whitney *U* test. NS: Not significant.

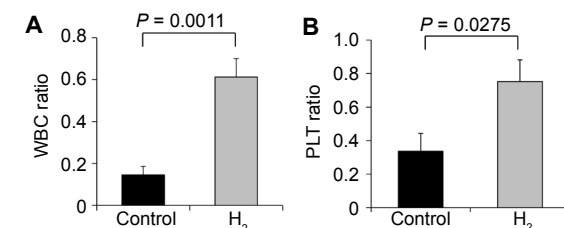


Figure 4: Effects of the hyperbaric hydrogen (H₂) gas treatment on the white blood cell counts (WBC, A), and platelet counts (PLT, B) of patients in control and H₂ groups

Note: WBC, and PLT were expressed as ratios of after to before the treatment. Data are given as the means ± SEM ($n = 7$ in control group, and $n = 16$ in H₂ group), and were analyzed by Mann-Whitney *U* test.



DISCUSSION

IMRT has been performed for cancer patients to alleviate the adverse effects associated with increased oxidative stress and inflammation¹; however, bone marrow damage still occurs in patients with multiple tumor lesions and large irradiation volumes. Thus, the development of safe and more effective technology is needed to mitigate further IMRT-induced bone marrow damage. H₂ was recently reported as a preventive and therapeutic antioxidant that selectively scavenges ·OH.⁵ The present study investigated whether H₂, a selective ·OH scavenger, mitigates IMRT-induced bone marrow damage in end-stage cancer patients, because selective and high concentrations of ·OH scavengers have potential as radioprotective agents. The patients received 5% H₂ gas or air for 30 minutes after each time of IMRT. These H₂ concentration and inhalation time were enough to demonstrate the mitigation effects, in the extrapolation based on the area under the curve from the H₂ concentration of blood or tissue in rats treated with H₂ gas.^{15,16} The results obtained demonstrated that the H₂ gas treatment alleviated IMRT-induced bone marrow damage without compromising the anti-tumor effects of IMRT. To the best of our knowledge, this is the first study to report the benefits of H₂ gas on IMRT in cancer patients.

Since the number of radiation times and total exposure doses of radiation need to be selected according to the size and number of tumors in individual patients, the number of radiation times performed in the present study ranged between a minimum of 5 (1 week) and maximum of 20 (4 weeks). Therefore, blood sampling to examine the adverse effects of radiation was performed from 1 to 4 weeks. However, the average number of radiation times and total exposure doses of radiation in the control and H₂ groups were equivalent, indicating that the design of the present study allowed for the protective effects of H₂ gas inhalation to be evaluated.

The present study demonstrated that the QOL, such as fatigue, depression, sleep, and gastrointestinal symptoms, was similar between the control and H₂ groups, and the inhalation of H₂ gas did not improve the QOL. However, since the main purpose of the present study was to mitigate radiation-induced damage, long-term inhalation may be needed to improve the QOL. Moreover, although the effects of H₂ gas inhalation were not examined in a non-irradiated control group (patients not undergoing radiotherapy), based on the findings of an animal study showing that H₂-rich saline did not affect hematological data in non-irradiated control mice,¹¹ H₂ gas inhalation may not have an impact on hematological data in patients.

The radioprotective effects of H₂ have been reported in different systems, including a cell-free system and various organs. In the cell-free system, Chuai et al.¹⁰ showed that the levels of ·OH produced by water radiolysis and the Fenton reaction were reduced by H₂ solution. Moreover, Yang et al.¹¹ demonstrated that a H₂-rich medium pretreatment decreased ·OH levels in AHH-1 cells, a human lymphocyte cell line. On the other hand, Yang et al.¹¹ also noted radiation-induced hematological changes in WBC and PLT, but not in RBC, HGB, or the mean corpuscular volume in mice subjected to total body radiation, and found that an intraperitoneal injection of H₂-rich saline significantly attenuated the depletion of

WBC and PLT. They also showed that H₂ reduced radiation-induced apoptosis in thymocytes and splenocytes in mice. These findings suggest that H₂ reduced radiation-induced ·OH levels by directly affecting ·OH levels, and simultaneously reduced radiation-induced oxidative stress, apoptosis, and inflammation by indirect effects on ·OH. In the present study, the H₂ gas treatment in combination with HCC therapy did not suppress the anti-tumor effects of IMRT in cancer patients because the response rates between the two groups were similar. This result is supported by the findings of Kang et al.¹² who demonstrated that the consumption of H₂-rich water reduced radiation-induced oxidative stress and the QOL in patients treated with radiotherapy for liver tumors without compromising anti-tumor effects. Therefore, the mechanisms underlying the radioprotective effects of H₂ gas may involve not only direct effects on ·OH, but also indirect effects on ·OH via the activation of the host-mediated antioxidant and anti-inflammation systems. These mechanisms have been supported by many papers reporting the radioprotective effects of H₂ in animal experiments.^{10,11,17-20}

In the present study, patients received the H₂ gas treatment after each time of IMRT. Previous studies reported the preventive (H₂ gas inhalation before radiation), but not therapeutic effects of this treatment. Furthermore, H₂ selectively scavenges ·OH and ONOO⁻.⁵ Since these reactive oxygen species are rapidly generated, however, many chemical agents generally exert stronger effects via prophylactic rather than therapeutic administration. Thus, the therapeutic administration protocol employed in the present study appears to accurately reflect the effects of H₂ gas inhalation because the mechanisms underlying the radioprotective effects of H₂ gas may involve not only direct, but also indirect effects on ·OH via the activation of host defense systems.

Hyperbaric oxygen therapy that involves housing patients for 1 hour in a chamber containing 100% O₂ at 2 atm (1 atm = 101.325 kPa) has been performed, which is effective for patients with decompression, peripheral circulation failure, wound dysfunction, and radiation-induced damage. In contrast, therapy using HCC is based on the principle of hyperbaric oxygen therapy, and patients are housed in a chamber containing 1.35 atm and air.² In the present study, based on Henry's law, patients in the H₂ group received 1.35-fold more H₂ and O₂ under 1.35 atm environmental conditions,²¹ which is equivalent to patients receiving 6.8% H₂ gas inhalation therapy under normal pressure. In additional experiments, the radioprotective effects of 6.5% H₂ gas inhalation therapy at normal pressure (data not shown) were found to be similar, suggesting the importance of inhaling higher H₂ concentrations to attenuate bone marrow damage. Although H₂ gas concentrations for the detonation limit in a mixture of H₂ and air are less than 4%, we recently demonstrated that the detonation limit was less than 10% in our previous experiment²² and a literature search.²³ Therefore, 6.5% H₂ gas therapy without HCC appears to be a clinically convenient, effective, and safe method for mitigating IMRT-induced bone marrow damage.

The development of safe and more effective radioprotective agents is very important in view of their potential application during radiotherapy for cancer patients. The radioprotective



effects of many synthetic and natural agents have been investigated in the past 50 years. Nutraceuticals, including vitamin C, vitamin E succinate, α -lipoic acid, and N-acetyl cysteine, in addition to hematopoietic growth factors and cytokines, such as stem cell factor, granulocyte colony-stimulating factor, granulocyte-macrophage colony-stimulating factor, and interleukin 3, have been reported to exert radioprotective effects in animal models.²⁴⁻²⁸ Amifostine, known as Ethyol or WR2721, is the only clinically accepted radioprotective agent, but is not considered to be a viable option as a radioprotective agent because of its inherent dose-limiting toxicities.²⁹⁻³¹ Although several drugs are in different stages of evaluation, none possess all of the requisite qualities of an optimum radioprotective agent. Thus, there are no safe and effective non-toxic radioprotective agents available for human use. H₂ has been reported to exert radioprotective effects in various animal models.^{10,11} Moreover, H₂ improves the QOL of patients treated with radiotherapy for liver tumors.¹² However, there is currently no definitive therapy to improve radiation-induced bone marrow damage in cancer patients. Therefore, the present study investigated whether H₂ gas mitigates IMRT-induced bone marrow damage in cancer patients, and the results obtained demonstrated that H₂ gas inhalation therapy with or without HCC alleviated IMRT-induced bone marrow damage without compromising the anti-tumor effects of IMRT. Clinical studies have demonstrated that H₂ has no adverse effects.⁷⁻⁹ Therefore, these findings may provide the foundation for a clinically applicable, effective, and safe strategy for a H₂ gas to mitigate IMRT-induced bone marrow damage.

Bone marrow damage, such as reductions in WBC (leukopenia) and PLT (thrombocytopenia), frequently occurs during cancer radiotherapy, including IMRT, and this is a limiting factor for radiotherapy.^{32,33} Since leukopenia and thrombocytopenia may cause infection and gastrointestinal bleeding, caution is required. If bone marrow damage may be attenuated by H₂ gas inhalation, it will lead to the prevention of infection and gastrointestinal bleeding. Therefore, H₂ gas inhalation may improve the prognosis of cancer patients.

In conclusion, the present study investigated whether H₂ gas inhalation mitigates IMRT-induced bone marrow damage in cancer patients. The results obtained demonstrated that H₂ gas inhalation therapy alleviated IMRT-induced bone marrow damage without compromising the anti-tumor effects of IMRT. However, this study had some limitations related to the number of patients, the retrospective observational analysis, and data collection from a single hospital. Although further large-scale clinical studies involving many hospitals are required, the present study suggests that this novel approach of H₂ gas inhalation therapy may be applicable to IMRT-induced bone marrow damage in cancer patients.

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Author contributions

SH and YA designed the study and analyzed the data; SH wrote the main text, analyzed the data, and prepared figures; YT, YA, XKL, NI, and ST supported this study by collecting data and giving advice. All

authors read and approved the final manuscript.

Conflicts of interest

The authors declare that they have no conflicts interests.

Financial support

None.

Institutional review board statement

The study was approved by an Ethics Committee Review of Tokyo Clinic and Research Institute ICVS Incorporated (Tokyo, Japan) on February 1, 2019, and was registered in the University Hospital Medical Information Network (UMIN) Clinical Trials Registry (UMIN ID: UMIN000035864) on February 20, 2019.

Declaration of patient consent

The authors certify that they have obtained patients' consent forms. In the form, patients have given their consent for their images and other clinical information to be reported in the journal. The patients understand that their names and initials not be published and due efforts will be made to conceal their identity.

Reporting statement

The writing and editing of the study report were performed in accordance with the STrengthening the Reporting of OBServational studies in Epidemiology (STROBE) statement.

Biostatistics statement

The statistical methods of this study were conducted and reviewed by the biostatistician of Clinic C4, Tokyo, Japan.

Author statement

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Data sharing statement

Datasets analyzed during the current study are available from the corresponding author on reasonable request.

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Additional files

Additional Table 1: Patient characteristics of control and H₂ groups.

Additional file 1: Hospital ethics approval (Japanese).

Additional file 2: Informed consent form.

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Additional Table 1 Patient characteristics of control and H₂ groups

No.	Sex	Age (yr)	Type of cancer		Stage
			Origin	Metastasis and/or dissemination	
Control					
1	F	69	Ovary	Multiple lymph node metastasis	IV
2	F	26	Stomach	Multiple bone and lymph node metastasis, right intrapelvic dissemination	IV
3	F	39	Colon	Multiple lung, liver and bone metastasis, right periovarian dissemination	IV
4	M	41	Lung	Multiple brain, bone, lymph node, subcutaneous and left adrenal grand metastasis, multiple pleural dissemination	IV
5	M	70	Prostate	Multiple bone and lymph node metastasis	IV
6	M	89	Prostate	Multiple bone and lymph node metastasis	IV
7	F	48	Breast	Multiple bone, lung and lymph node metastasis	IV
H ₂					
1	F	67	Ovary	Multiple lymph node metastasis	IV
2	M	55	Prostate	Multiple bone and lymph node metastasis	IV
3	M	67	Prostate	Multiple bone and lymph node metastasis	IV
4	M	78	Prostate	Multiple bone, lung and lymph node metastasis	IV
5	M	62	Ureter	Multiple lung and lymph node metastasis	IV
6	F	49	Liver	Multiple lung and lymph node metastasis, peritoneal dissemination	IV
7	F	82	Liver	Multiple lung, liver and lymph node metastasis	IV
8	M	82	Stomach	Multiple lung, liver and subcutaneous metastasis, peritoneal dissemination	IV
9	F	73	Pancreas	Multiple bone and lung metastasis, peritoneal dissemination	IV
10	M	82	Bile duct	Multiple bone metastasis	IV
11	M	69	Pancreas	Multiple lung, bone, liver and lymph node metastasis,	IV

				peritoneal dissemination	
12	F	68	Pancreas	Multiple lung and lymph node metastasis, subcutaneous and right adrenal grand metastasis, peritoneal dissemination	IV
13	F	58	Ovary	Multiple lymph node metastasis, peritoneal dissemination	IV
14	F	48	Breast	Whole meninges dissemination	IV
15	F	79	Urinary bladder	Multiple bone and lymph node metastasis	IV
16	M	35	Urinary bladder	Multiple bone, lung and lymph node metastasis, left adrenal grand metastasis	IV

Note: F: Female; M: male. The stage of the patients was confirmed according to the TNM classification.in Union for International Cancer Control.