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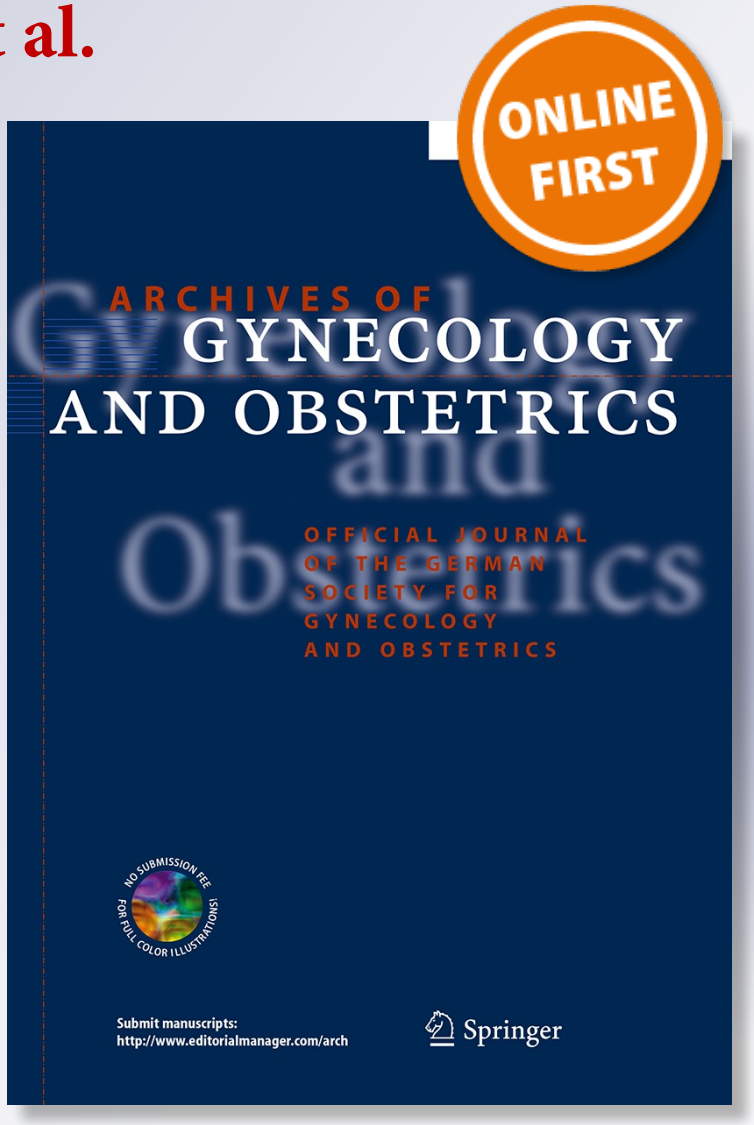


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GYNECOLOGIC ONCOLOGY

# Validation of the distress and impact thermometer and the changes of mood during the first 6 months of treatment in gynecological cancer patients: a Kansai Clinical Oncology Group (KCOG)-G1103 prospective study

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**Abstract**

*Purpose* To verify distress and impact thermometer (DIT) for screening emotional distress in gynecological cancer patients by Hospital Anxiety and Depression Scale total (HADS-T) as gold standard and to assess emotional changes by DIT and HADS-T.

*Methods* A prospective study was conducted in newly diagnosed gynecological cancer patients during the peri-treatment period after the cancer diagnosis followed by 6-month. We defined a HADS-T score of ≥11 as being indicative of emotional distress.

*Results* 117 patients were enrolled between May 1, 2011 and March 31, 2012, and 95 were eligible. The median age was 54 years (range 31–77). (1) From the baseline to 3-month, distress (DIT-D) ≥4 with Impact (DIT-I) ≥2 exhibited sensitivity, specificity, positive predictive value (PPV), and negative predictive values (NPV) of 0.776 [95 % confidential interval (CI) 0.688, 0.850], 0.889 (95 % CI 0.824, 0.954), 0.868 (95 % CI 0.792, 0.949), and 0.808 (95 % CI 0.731, 0.886), respectively. (2) At 6-month, DIT-D ≥2 with DIT-I ≥1 exhibited sensitivity, specificity, PPV and NPV of 0.893 (95 % CI 0.778, 1), 0.825 (95 % CI 0.707, 0.942), 0.781 (95 % CI 0.638, 0.928), and 0.917 (95 % CI 0.826, 1). (3) At 6-month, the HADS-T, DIT-D, and DIT-I scores in individual patients were significantly reduced by a mean of 4.57 ($p < 0.0001$), 2.34 ($p < 0.0001$), and 1.10 ($p = 0.0031$), respectively, compared with those scores of baseline (Student's paired *t* test), but still remained high.

*Conclusions* (1) On acute phase within 3-month setting, DIT; DIT-D ≥4 with DIT-I ≥2, is a reliable cut-off to screen emotional distress among gynecological cancer patients. (2) The patients' moods had improved, but not completely recovered at 6-month after the diagnosis.

**Keywords** Gynecological malignancy · Emotional distress · DIT · HADS

**Electronic supplementary material** The online version of this article (doi:10.1007/s00404-016-4166-y) contains supplementary material, which is available to authorized users.

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## Introduction

Almost a half of patients who are diagnosed with cancer develop anxiety or depressive disorders [1, 2], and 58 % of cancer patients are reported to feel anxious [3]. Above all, gynecologic cancer patients are reported to have one of the highest levels of anxiety and depression [4]. But oncologists often underestimate the distress derived from cancer in daily practices [5, 6]. Some oncologists attempt to perceive the emotional distress, but they often

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end unsuccessfully by trying to apply unfamiliar psychiatric assessments [6]. Hence, screening tools that enable oncologists to evaluate the extent of the emotional distress have been devised. The Hospital Anxiety and Depression Scale (HADS; a 14-item self-reported questionnaire) is one of the most well-validated screening tools for emotional distress in cancer patients and assesses anxiety and depression [7, 8]. However, it is not widely employed, except in clinical research, because it takes a lot of efforts for busy oncologists to use [8–11]. Therefore, an easier-to-use screening tool is desired. The distress and impact thermometer (DIT) consists of a 2-item [questions about the severity of the patient's distress (DIT-D) and its impact (DIT-I)] self-reported questionnaire and is easy to administer (Fig. 1). Patients are asked "Please circle the number (0–10) that best describes how much distress you have been experiencing in the past week including today" for DIT-D and "Please choose the number (0–10) that best describes how much "impact" caused by the distress you have been experiencing on daily life activity" for DIT-I [9]. In response to each question, the patient simply circles the appropriate number on an 11-point rating scale. Higher scores indicate a less favorable status. Thus, the DIT is very simple to use, but has not been validated sufficiently [9, 10] in gynecologic cancer patients.

## Aims of the study

First, to validate the utility of DIT for detecting emotional distress in newly diagnosed gynecologic cancer patients and to identify the optimal DIT-D and DIT-I cut-off points for the distress in this setting with HADS-T; total HADS score as a gold standard. Second, to study the emotional changes in individual patients of the gynecologic cancer patients from the diagnosis to the first 6-month assessed by means of HADS-T and DIT.

## Patients and methods

To be eligible for this study, the patients had to (1) have histologically confirmed newly diagnosed gynecological cancer, (2) be scheduled to undergo treatment, (3) have a life expectancy of at least 6 months, and (4) give informed consent in written form. This study required that the patients must be registered prior to the commencement of

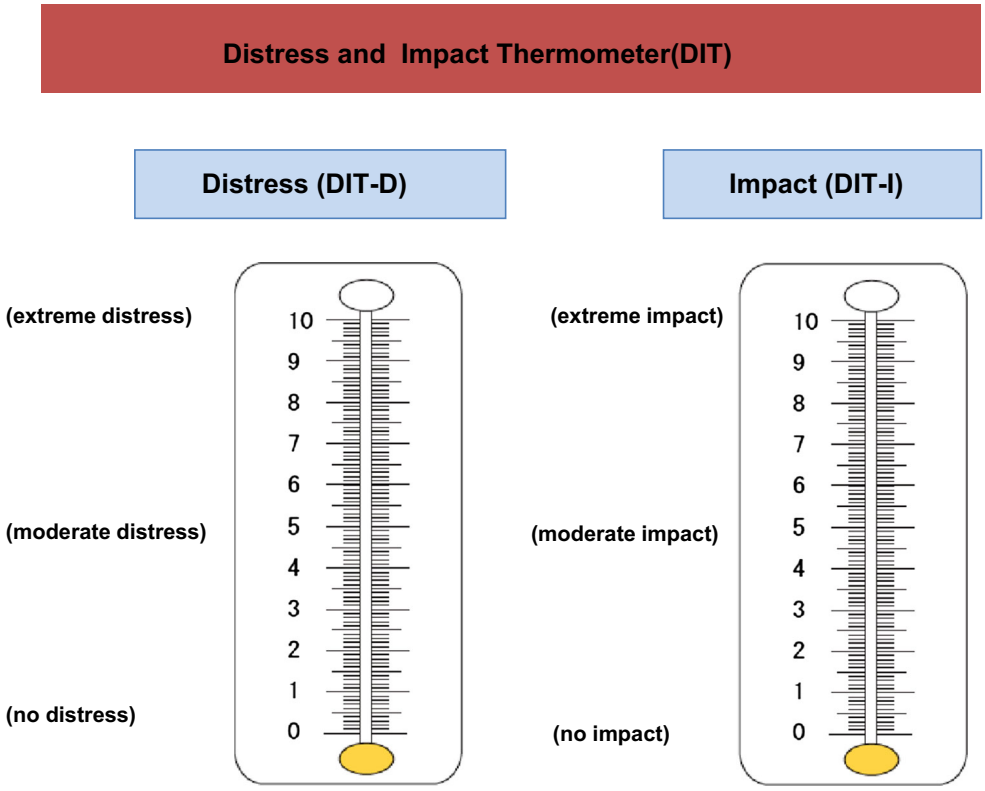


**Fig. 1** DIT; Distress and Impact Thermometer. Patients are asked to circle the number (0–10) that best describes how much distress you have been experiencing in the past week including current day for distress (DIT-D) and followed to answer the number (0–10) that best describes how much "impact" caused by the distress you have been experiencing on daily life activity for impact (DIT-I). Adapted Akizuki et al. [9]

treatment. After informed consent had been obtained, the registered participants were requested to fill out the questionnaires (HADS and DIT) during peri-treatment period before treatment had begun (pretreatment; baseline), at 3 months (3-month), and at 6 months (6-month) from the treatment had begun. If a suspected ovarian cancer turned out to be benign or was demonstrated not to be a gynecological origin by the final pathological diagnosis, the patient was withdrawn from the study, even though they had been provisionally registered. If the enrolled patients were recurred, they were required to respond to no further questionnaires at that point. A HADS-T score of ≥11 is considered to be indicative of positivity for psychological distress at a severity level of adjustment disorder or major depression, as is validated in Japan, [8] and is also recommended to screen mental disorder [12]. We used receiver operating characteristic (ROC) curve analysis of DIT-D and DIT-I score combinations to determine the optimal cut-off points, i.e., those that yielded the highest sensitivity—(1-specificity) values, for distinguishing distressed and anxious patients with HADS-T ≥11. Differences in individual patients' HADS-T, DIT-D, or DIT-I scores among the three examined time points were also evaluated with the Student's *t* test for paired data. The participants were recruited at four institutions belonging to the Kansai Clinical Oncology Group (KCOG), Osaka, Japan (Electronic Supplementary Material Table 1). This trial was conducted in accordance with the Declaration of Helsinki, good clinical practice guidelines, and applicable laws and regulations and received ethical approval from the review boards of the KCOG and each institution. If the patients desired to consult a psychiatrist, their intension was respected. This protocol was registered in the University Hospital Medical Information Network Clinical Trials Registry (UMIN000005727).

### Sample size and statistical analysis

We assumed that mean HADS points are 8.4 with a standard deviation of 0.4, and mean DT points are 4.3 with a standard deviation of 0.3 [13, 14]. Because, to detect a difference in longitudinal data between pretreatment (baseline) and 3 months, between 3 and 6 months, and between pretreatment (baseline) and 6 months, we estimated that 38 patients were needed to provide 80 % power at the 5 % significance level by paired *t* test. In consideration of decline during 6-month-observation, we planned to recruit for a total of 100 participants. And if questionnaires were unreturned during the surveillance, we did not substitute missing data and excluded them at that period from the analysis.

The data were analyzed using the statistical software JMP version 11.1.1J (Tokyo, Japan).

## Results

117 patients were enrolled between May 1, 2011 and March 31, 2012, and 95 were eligible for inclusion and participated in the study. 11 patients were excluded because their first questionnaires were filled in after the treatment had begun, 10 patients were excluded because they did not send the first questionnaire back after the consents, and one patient was excluded because her suspected ovarian malignancy subsequently turned out to be a metastatic ovarian cancer in the exploratory surgery. The number of completed questionnaires (HADS-T and DIT) at pretreatment (baseline), 3-, and 6-month was 95, 80, and 72; 4 patients answered only HADS-T at 6-month, respectively (Fig. 2). The characteristics of the study participants are shown in Table 1. None of the patients suffered relapses during the study period. There were no significant factors such as age, performance status, numerical rating scales for somatic pain, and scores of former inquiry of HADS-T, DIT-D, and DIT-I in completing the series of questionnaires. HADS-T are composed of 2 components of depression (HADS-D) and anxiety (HADS-A). We preliminarily studied correlations of HADS-T, HADS-D, and HADS-A. Spearman's rank correlation coefficient and the corresponding *p* values between HADS-T and HADS-D, between HADS-T and HADS-A, and between HADS-D and HADS-A were 0.94, 0.94 and 0.764, respectively; $p < 0.0001$, 0.0001, 0.0001, respectively (Electronic Supplementary Material Fig. 2). Cronbach's alpha for all the sets was 0.912. For the integrated assessment of emotional distress, HADS total would be appropriate.

The area under the ROC curve values for each DIT-D and DIT-I with respect to HADS scores of ≥11 were 0.872 and 0.870, respectively. The sensitivity, specificity, PPV, and NPV, sensitivity—(1-specificity), positive likelihood ratio (PLR), and negative likelihood ratio (NLR) for various combinations of DIT-D with DIT-I scores are shown in Electronic Supplementary Material Table 2. Combination of DIT-D ≥4 with DIT-I ≥1 was the highest for sensitivity—(1-specificity) of 0.625 in all the recruited periods. We examined cut-off point with HADS total ≥11 in a post hoc subgroup of the acute period between the baseline and 3-month, the threshold revealed DIT ≥4 with DIT-I ≥2. As in the subacute period of 6 months, the cut-off point was DIT ≥2 with DIT ≥1. Between the baseline and 3-month, which we call for acute phase, combination of DIT-D ≥4 with DIT-I ≥2 was the highest for sensitivity—(1-specificity) of 0.665.

The frequencies of HADS-T scores of ≥11 at baseline, 3-month, and 6-month were 58 % (55/95), 38 % (30/80), and 40 % (28/72), respectively. DIT-D ≥4 and DIT-I ≥2 at

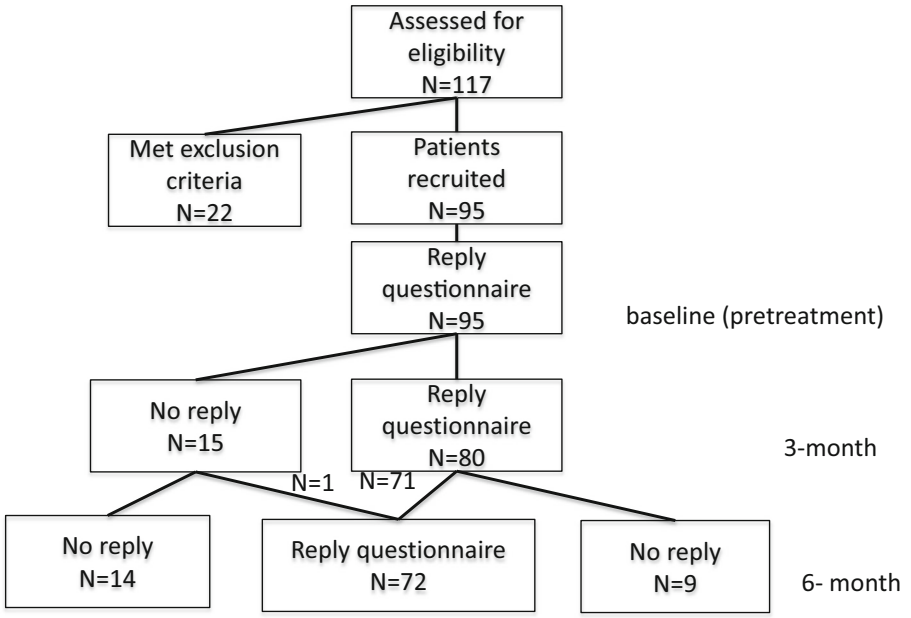


**Fig. 2** Flowchart of the number of patients assessed at each time of the study

baseline, 3-, and 6-month were 52 % (50/95), 32 % (26/80), and 20 % (14/68), respectively. At 6-month, "sensitivity—(1-specificity)" maximizes up to 0.718 under DIT-D≥2 with DIT-I≥1 and the frequency for DIT revealed 41 % (28/68). Emotional distress evaluated by DIT might differ from that by HADS in subacute setting. The mean score for each period is shown in Tables 2 and 3. At 3-month after the start of treatment, the HADS-T and DIT-D scores in individual patients were significantly reduced by a mean of 3.13 ($p < 0.0007$) and 1.46 ($p < 0.0001$), respectively, compared with the baseline score. Though the DIT-I score was also reduced by a mean of 0.24, the decrease was not significant ($p = 0.546$) (Student's paired $t$ test). At 6-month, the HADS-T, DIT-D, and DIT-I scores in individual patients were all significantly reduced by a mean of 4.57 ($p < 0.0001$), 2.34 ($p < 0.0001$), and 1.10 ($p = 0.0031$), respectively, compared with the baseline scores (Student's paired $t$ test) (Table 3).

## Discussion

Distress is perceived to have a multifactorial unpleasant emotional experience of a psychological (i.e., cognitive, behavioral, emotional), social, and/or spiritual nature that may interfere with the ability to cope effectively with cancer, its physical symptoms and its treatment. Distress extends along a continuum, ranging from common normal feelings of vulnerability, sadness, and fears to problems that can become disabling [15]. Several components of distress would be augmented in peculiar context caused by physical and/or psychological status. And the magnified domains express diverse symptoms; depression, anxiety, panic, social isolation, and existential and spiritual crisis. In cancer patients, such depressive states during treatment can lead to further problems such as an increased risk of suicide [16], reduced treatment adherence [10], and a general derogation of quality of life [17]. Hence, psychosocial counseling is essential for cancer patients [18]. But oncologists frequently focus solely on the disease they are treating and tend to pay little attention to patients' anxiety and concerns. To bridge a gap in recognizing emotional distress between patients and oncologists [5, 19, 6], many self-administered questionnaire-based screening tools have been developed. That enables oncologists to understand cancer patients' emotional distress more properly [12, 20–22]. However, long measures, such as Profile of Mood States: 37-item scale to assess six mood states (tension–anxiety, depression–dejection, anger–hostility, confusion–bewilderment, fatigue–inertia, and vigor–activity), Beck symptom inventory, or General Health Questionnaire-28, could assess multiple domains and are highly reliable with cost. HADS has less number of questionnaires and observes anxiety and depression principally. HADS is one of the most well-validated tools for screening for emotional distress in cancer patients [14, 23, 24], but scoring HADS still requires additional work, which is an obstacle to its use in clinical practice. Distress thermometer (DT) is one-phrase questionnaire for screening of distress and is easy to score with cut-off point of which is recommended ≥4. And DT measures the broader concept of distress, without giving specific information on the probability of psychiatric morbidity [21]. DT demonstrates a sensitivity range of 0.5–1.0 with a median of 0.83, and the

**Table 1** Patients' characteristics ($N = 95$)

| | No. of patients (%) |
|---|---|
| Age (years) | Median: 54 (range 31–77) |
| Marital status | |
| Married/living together | 69 (72 %) |
| Single/divorced/widowed | 25 (26 %) |
| Unknown | 1 (1 %) |
| Having children | |
| No | 20 (21.5 %) |
| Yes | |
| ≥20-year-old | 48 (51.6 %) |
| <20-year-old | 25 (26.9 %) |
| Cancer site | |
| Uterine cervix | |
| Stage I or II | 34 (36.1 %) |
| Stage III or IV | 2 (3.2 %) |
| Uterine corpus | |
| Stage I or II | 31 (32.9 %) |
| Stage III or IV | 2 (2.1 %) |
| Ovarian and others | |
| Stage I or II | 17 (18 %) |
| Stage III or IV | 6 (6.3 %) |
| Treatment received | |
| Surgery alone | 43 (48.4 %) |
| Surgery and CT (or CCRT) | 45 (47.4 %) |
| CCRT | 5 (5.3 %) |
| CT | 1 (1.1 %) |
| Suffered previous disease | |
| Malignant disease | 9 (9.5 %) |
| Other disease | 36 (37.9 %) |
| Having blood relatives of the fourth degree or closer who had developed malignant disease | 45 (47.4 %) |
| Duration of the initial treatment (days) | |
| <90 | 57 (60 %) |
| ≥90 | 38 (40 %) |
| Psychotherapy/psychiatric treatment | |
| Psychiatric consultation | 2 (2 %) |
| Anti-depressants, hypnotic, or group therapy | 11 (12 %) |

*CT* chemotherapy, *CCRT* concurrent chemoradiotherapy

**Table 2** Changes in the patients' HADS-T, DIT-D, and DIT-I scores

| | Pretreatment, mean (95 % CI) | 3 months after diagnosis, mean (95 % CI) | 6 months after diagnosis, mean (95 % CI) |
|---|---|---|---|
| HADS total | 12.7 (11.1, 14.3) | 10.2 (8.5, 11.8) | 8.9 (7.2, 10.6) |
| DIT-D | 4.8 (4.2, 5.5) | 3.3 (2.7, 3.9) | 2.6 (1.9, 3.2) |
| DIT-I | 3.1 (2.5, 3.7) | 2.6 (2.0, 3.2) | 1.9 (1.3, 2.5) |

The Student's paired *t* test was used to assess the significance of differences

*HADS* Hospital Anxiety and Depression Scale, *DIT* Distress and Impact Thermometer, *CI* confidence interval

**Table 3** Comparisons between the scores observed in each period

| | Comparison | Mean difference | *p* |
|---|---|---|---|
| HADS total | 3 months–pretreatment | −3.13 | <0.0007* |
| | 6 months–pretreatment | −4.57 | <0.0001* |
| | 6 months–3 months | −1.43 | 0.0196* |
| DIT-D (distress) | 3 months–pretreatment | −1.46 | <0.0001* |
| | 6 months–pretreatment | −2.34 | <0.0001* |
| | 6 months–3 months | −0.88 | 0.0035* |
| DIT-I (impact) | 3 months–pretreatment | −0.24 | 0.548 |
| | 6 months–pretreatment | −1.10 | 0.0031* |
| | 6 months–3 months | −0.87 | 0.0044* |

*HADS* Hospital Anxiety and Depression Scale, *DIT* Distress and Impact Thermometer, *CI* confidence interval

* Statistically significant ($p \leq 0.05$)

specificity ranged from 0.36 to 0.95 with a median of 0.68 [25]. DIT is designed to screen emotional distress more precisely by adding impact component (DIT-I) to DT and to preserve easy-to-administer. There is another screening tool for distress adding four more domains to DT (DIT-D); distress, anxiety, depression, anger, and need for help [26]. It is hard to deal with high reliability and simplicity. And what is more, long questionnaires could not be answered readily for the patients soon after the bad news. Anxiety and depression occur early after the cancer notification and that is the reason why we focused on DIT. Gynecologic cancer patients have one of the highest levels of anxiety and depression [4], but there are no studies on verifying screening tools to detect emotional distress for gynecologic cancer patients. Therefore, we conducted this study and confirmed that DIT; DIT-D ≥4 with DIT-I ≥2 is validated to screen emotional distress of gynecological malignant patients who are newly diagnosed and treated, within acute phase sensitivity—(1-specificity): 0.665. Meanwhile, Akizuki et al recommended DIT-D.4 with DIT-I.3 was the cutoff points with sensitivity; 0.82 and specificity; 0.82 [9] in a variety of the treatment statuses with non-gynecological malignant patients. DIT-I cut-off ≥2 in this study might be lower point setting than the Akizuki's report and lowered power to identify distressed patients deteriorated with sensitivity 0.776 (95 % CI 0.688, 0.850). But screening power outstrips the former with specificity: 0.889 (95 % CI 0.824, 0.954) and with NPV: 0.808 (95 % CI 0.731, 0.886). Baken et al. [10] also suggested that adding DIT-I to DIT-D is more accurate than single use of DIT-D or DIT-I. Martinez et al. [27] reported that adding DIT-I could not improve the accuracy of identifying distress. Perception of how each patient feels mentally and emotionally could be differed in various backgrounds, such as language, country, clinical setting, sample characteristic, linguistic situation, and culture, influence the screening accuracy [25, 27, 28]. Answering posture to the questionnaires would also be transformed into various manifestations at times [4, 29]. In gynecological malignancy-specific with early on initiation of treatment, DIT-D.4 with DIT-I.2 is the best cut off points at the acute phase of the disease.

Although it seems that the participants' HADS-T, DIT-D, and DIT-I scores decreased during the study period, it was noteworthy that the participants' mean HADS score was 8.9 at 6 months after the start of treatment, which was still high (Tables 2, 3). We could not detect dominant factors, but it might be possible that some participants who did not return the questionnaires felt low to respond. Therefore, oncologists should pay attention to patients' distress not only in peri-treatment period but also after the successful completion of planned treatment without relapse. There are reports that the scores remain high within 6-month-assessed DT or HADS [13, 14]. In this study, the emotional state of gynecological cancer patients was researched over the first 6 months of the treatment, and we confirmed that the patients' psychological condition got better during the research over time. Though the patients' DIT-D scores were significantly decreased at 3 months after the start of treatment, the DIT-I scores for the same period did not decreas significantly from the baseline. We also have to remember the fact that 38 (40 %) of the participants were still receiving their initial round of treatment at 3 months after the start of treatment as one of the causes. Cancer treatment may make participants feel unwell, which places an emotional burden on them. This might be attributed, in part, to no significant decrease in DIT-I score at 3-month. The patients, soon after the diagnosis, gradually resume mental stabilities, but 6-month-elapse of peri-treatment period is insufficient for them to get restored from the distressed status. All the participants were free from cancer relapse, 25–40 % patients at 6-month were still suffering from the distress. Hence, it will take about at least 1 year to reserve stable mental status [13].

Observation period we had planned in the study was too short to confirm the recovery from distress. By adding the impact component of suffering on daily living, DIT could uncover psychosocial concerns to a certain degree. Cut-off point of DT is ranged at 2–7 [10, 12, 25, 27, 29, 30], and specificity ranged at 0.36–0.85 under the condition. Our specificity for DIT-D ≥4, 0.823, is on par with former papers. And sensitivity, specificity, PPV, and NPV are high. But DIT-I decreased gradually in our study. Hence, we think that DIT-I, impact on daily living, turned out to be not easily overcome. This is a critical point that other various aspects of distress might be represented as of DIT-I. Though single-item screening in a cancer care setting may not adequately capture clinical anxiety and depression, single items assessing patients' perceived levels of anxiety and depression could be practical indicators of their needs for psychosocial care [31]. Distress takes cancer patients out of the ability to express their severity. Many patients are reluctant to disclose psychosocial concerns, viewing them as inevitable or insoluble or not wishing to burden healthcare staff [32].

From our study, DIT has an efficacy, especially, in acute phase. Therefore, DIT might be recommended to be used in acute phase, when patients are in both psychological stress and physiologically bad conditions. And because DIT is composed of short questionnaires and almost distress-containing components and considering troubles in daily living, it is one of the optimal screening tools with acute psychologically and physiologically distressed patients. In acute phase, most cancer patients have the tendency to be less concentrated and not to answer the wide-ranging questionnaire well. We do not think that long measure tools always work well and seize a sense of patients' feelings. Hence, we think that short questionnaire containing impact component on daily living is an optimal screening tool.

The main limitation of this study is that we used the HADS-T as a gold standard for emotional assessments. There are five visual analog scales in the form of four predictor domains (distress, anxiety, depression, anger, and one outcome domain; need for help) which might be required to perceive the whole emotional distress [26]. Therefore, we should understand that efficacy of brief instruments might be sparse and less reliable. Meanwhile, psychological distress in cancer patients refers more specifically to anxiety, depression, and adjustment disorders [33]. We should pay attention to anxiety and depression of the newly notified cancer patients with special attention. Although the HADS is one of the most widely used self-administered questionnaires for assessing cancer-induced depression [12, 20–22, 34, 35], there are some differences in the diagnosis of adjustment disorder or depression between the HADS classification and psychiatric interview analyses based on the DSM-IV. HADS-T optimal thresholds might vary, but there are validation studies on DIT with HADS-T ≥11 as positive [34–36]. Early on notification of cancer within the 3 months, distress possesses distinctive features of anxiety and depression, and these could be well determined by HADS perceptively. DIT-D (DT) cut-off score ≥4 [14] is also recommended for detecting cases in distress [14, 34]. In gynecological cancer patients, we also confirm that DIT-D ≥4 was adequate in the acute period from the baseline to 3-month. But in the subacute period at 6-month, DIT-D ≥2 was relevant cut-off score. DIT-D (DT) ≥2 is also reported to be best cut-off for clinical use at 6 months after diagnosis according to HADS subscale ≥8 [37]. It is similar to our result that cut-off for DIT-D is lowered in subacute phase compared with HADS.

We think that dissociation between HADS and DIT could arise as distress varies by time course. If we, especially, would have intended to screen anxiety and depression components in subacute phase, it might have been possible to use DIT-D (DT) ≥2 with asking a single yes/no question "does the distress you have been experiencing have any impact on daily life activity-yes/no" for DIT-I ≥1. We presume that both DIT-D and DIT-I might offer different perspectives of distress other than anxiety and depression affected by physical and/or psychological changes. In another finding, there is a report that negative screens are wrongly lead patients as having no needs for psychiatric help and that positive screens are always interpreted to accept meddlesome psychological help beyond patient's inclinations [38]. We surely attach great importance to that point. But oncologists always confront patients' distress firsthand. Whether these screenings for cancer patients would not reflect real needs for psychiatric help, we believe that a clue to alleviate patients' emotional distress is to use screening tools in daily clinical practice and to get a sense of psychological stress of the patients. We still believe that it would be beneficial to a number of patients if oncologists were able to evaluate emotional distress and administer early appropriate management [13, 14, 28, 20].

And oncologists could lead to patients being referred to psycho-oncologists and would be able to intervene to alleviate patients' emotional distress by the most effective way of detecting psychologically distressed patients in daily clinical practice [24, 25]. Communication skills training could also help oncologists to lessen the emotional pain caused by cancer [39, 40]. We would like to insist that the DIT is an excellent tool for making such assessments in gynecological cancer patients during the period from diagnosis to follow-up. We recommend using of the DIT; DIT ≥4 with DIT-D ≥2 for screening emotional distress of gynecological cancer patients from the diagnosis to follow-up period of at least 3-month or at acutely stressed

situation, DIT use after a long time from the bad news to confine anxiety and depression, cut-off point should be lowered to DIT-D $\geq$2 with DIT-I $\geq$1.

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**Compliance with ethical standards**

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**Conflict of interest** All authors declare that they have no conflict of interest.

**Ethical approval** All procedures performed in studies involving human participants were in accordance with the ethical standards of each institutional review board and KCOG institutional review board with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

**Informed consent** Informed consent was obtained from all individual participants included in the study.

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