# **Clinical Psychopharmacology and Neuroscience - Manuscript**

# **Submission**

- Manuscript ID: CPN-20-824
- Title: Associations of serum serotonin levels with 12-week and 12-month remission in patients with depressive disorders
- Running Title: Serum serotonin for depression remission
- Article Type: Original Article
- KeyWords: depression, remission, serotonin, antidepressant, treatment strategy

### 1 Abstract

*Objectives:* To investigate associations between baseline serum serotonin levels and short- and
 long-term treatment outcomes in outpatients with depressive disorders in a naturalistic one year prospective study design.

5 Methods: Patients were recruited at a University hospital in South Korea from March 2012 to 6 April 2017. At baseline, blood samples were obtained from 1,094 patients who received initial antidepressant monotherapy (Step 1). After the Step 1 treatment, further treatment steps (at 7 least Steps 2–4) could be administered every 3 weeks during the acute treatment phase (3, 6, 9, 8 9 and 12 weeks; N = 1.086), and every 3 months during the continuation treatment phase (6, 9, and 12 months; N = 884). In cases showing an insufficient response or intolerable side effects, 10 11 patients were asked to choose whether to remain at the current step or enter the next treatment step, with alternative strategies including switching, augmentation, combination, and a mixture 12 of these approaches. Remission was defined as a Hamilton Depression Rating Scale score of  $\leq$ 13 14 7.

15 *Results:* The remission group had significantly higher baseline serum serotonin levels among 16 patients who received Step 1 monotherapy in both acute and continuation treatment phases. 17 These associations remained significant after adjustment for relevant covariates. No 18 associations were found with any other treatment steps.

19 Conclusions: Baseline serum serotonin levels may be used as a biomarker for predicting short-20 and long-term treatment outcomes in antidepressant monotherapy-treated patients with 21 depressive disorders in a real-world clinical setting.

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23 Key Words: depression; remission; serotonin; antidepressant; treatment strategy.

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### 1 1. Introduction

2 Depressive disorders are one of the most serious public health problems globally, affecting 3 more than 250 million people worldwide (1, 2). Antidepressants are the first-line treatment, particularly for moderate to severe major depressive disorder (MDD) (3). Currently used 4 5 antidepressants have similar efficacy to each other and are associated with remission rates of 6 less than one third of patients after 8~12 weeks of first trial (2-5). Considering its high disease 7 burden and low initial antidepressant remission rate, it is necessary to develop biomarkers that 8 predict antidepressant outcomes that could aid in deciding personalized treatment strategies in 9 depressive disorders. Based on the main hypotheses pertaining to the biological mechanisms 10 of depression, such as the monoamine transmitter hypothesis (6), the inflammatory hypothesis 11 (7), the neurotrophic hypothesis (8), and the HPA axis dysfunction hypothesis (9), efforts have been made to develop peripheral biomarkers of antidepressant response. However, clinically 12 meaningful biomarkers for differentiation between treatment strategies have not yet been 13 14 developed (10).

Although the detailed pharmacological mechanisms of antidepressants remain unclear, most types of antidepressants are thought to upregulate serotonin (5-hydroxytryptamine, 5-HT) levels in the synaptic cleft (11). 5-HT is a monoamine neurotransmitter that regulates various physiological functions in the central and periphery (gastrointestinal tract, cardiovascular system, immune system, endocrine system, etc.). By acting as a neurotransmitter in the central nervous system (CNS), 5-HT regulates mood (12), sleep-wake behavior (13), and appetite (14).

Taking into account the proposed mechanism of action for antidepressants, there have been efforts to explore peripheral blood 5-HT as a biomarker for antidepressant response. Recent studies have shown that higher baseline plasma 5-HT levels before selective serotonin reuptake inhibitor (SSRI) treatment were associated with a better treatment response (15, 16).

1	They hypothesized that these associations might be related to 5-HT <sub>1A</sub> autoreceptor
2	hypofunctioning (17, 18). In the CNS, 5-HT negatively regulates the activity of the 5-HT
3	system through 5-HT <sub>1A</sub> autoreceptor signaling (19). Because 5-HT <sub>1A</sub> autoreceptor
4	hypofunctioning leads to increased 5-HT in the synapse and is associated with a better
5	antidepressant response (17, 18), they speculated that increased pre-treatment plasma 5-HT
6	levels might be related to 5-HT <sub>1A</sub> autoreceptor hypofunctioning. Since these studies evaluated
7	the associations between 5-HT levels and treatment responses for up to 8 weeks in MDD
8	patients treated with SSRIs, associations between 5-HT levels and long-term outcomes in
9	patients using various types of antidepressant in real-world clinical situations are not known.
10	In the present study, by using data from a naturalistic prospective study of Korean
11	patients with depressive disorder, we investigated associations between baseline serum 5-HT
12	levels and short- and long-term remission in patients using various treatment strategies based
13	on early clinical decisions.
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### 1 **2. Methods**

### 2 2.1. Study outline

3 This study was carried out as a component of the MAKE Biomarker discovery for Enhancing antidepressant Treatment Effect and Response (MAKE BETTER) program. Details of the 4 5 study have been published as a design paper (20) and registered with cris.nih.go.kr (identifier : 6 KCT0001332). Data on socio-demographic and clinical characteristics were obtained using a structured clinical report form (CRF) by clinical research coordinators, who were blind to 7 8 treatment modalities. They were trained in CRF implementation and data collection methods 9 by the research psychiatrists. Patients' data were recorded on a CRF, registered in the website 10 of the MAKE BETTER study 11 (http://icreat.nih.go.kr/icreat/webapps/com/hismainweb/jsp/cdc n2. live) within 3 days, and monitored by data management center personnel. This study was approved by the Chonnam 12 National University Hospital Institutional Review Board (CNUH 2012-014). 13

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### 15 2.2. Participants

Patients with depressive disorders were consecutively recruited from March 2012 to April 2017 16 from those who had visited the outpatient psychiatric department of Chonnam National 17 18 University Hospital. Research psychiatrists assessed and diagnosed depressive disorders using 19 the Mini-International Neuropsychiatric Interview (MINI) (21), a structured diagnostic 20 psychiatric interview based on the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM- IV) criteria. As the aim of the study was to reflect a real-world clinical 21 22 setting as closely as possible, broad inclusion criteria and minimal exclusion criteria were applied. Inclusion criteria were: i) aged older than 7 years; ii) diagnosed with MDD, dysthymic 23 disorder, or depressive disorder not otherwise specified (NOS); iii) Hamilton Depression 24

Rating Scale (HAMD) (Hamilton, 1960) score  $\geq$  14; iv) able to complete questionnaires, 1 2 understand the objective of the study, and sign the informed consent form. Exclusion criteria 3 were as follows: i) unstable or uncontrolled medical condition; ii) unable to complete the psychiatric assessment or comply with the medication regimen, due to a severe physical illness; 4 5 iii) current or lifetime DSM-IV diagnosis of bipolar disorder, schizophrenia, schizoaffective 6 disorder, schizophreniform disorder, psychotic disorder NOS, or other psychotic disorder; iv) 7 history of organic psychosis, epilepsy, or seizure disorder; v) history of anticonvulsant 8 treatment; vi) hospitalization for any psychiatric diagnosis apart from depressive disorder (e.g., 9 alcohol/drug dependence); vii) electroconvulsive therapy received for the current depressive episode; viii) pregnant or breastfeeding. All participants reviewed the consent form and written 10 informed consent was obtained. For participants aged under 16, written consent was obtained 11 12 from a parent or legal guardian, and written assent was obtained from the participant.

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### 14 **2.3. Measurements at baseline**

Socio-demographic characteristics obtained comprised age, gender, years of formal education, 15 16 marital status (currently married or not), cohabiting status (living alone or not), religion 17 (religious observation or not), occupation (current employed status or not), and income (above or below 2,000 USD). Clinical characteristics evaluated were comprised of diagnoses of 18 19 depressive disorders as mentioned above with certain specifiers, age at onset and duration of 20 illnesses, history of previous depressive episodes (recurrent or first episode), number of previous depressive episodes, duration of present episode, family history of depression, history 21 22 of suicide attempt, and number of concurrent physical disorders (applying a questionnaire 23 enquiring about 15 different systems or disorders). Assessment scales for investigating 24 symptoms and function were administered. Depressive symptoms were evaluated by the

HAMD, anxiety symptoms by of the Hospital Anxiety Depression Scale-anxiety subscale
(HADS-A) (22), quality of life by the EuroQol-5D (EQ-5D) (23), and functioning levels by the
Social and Occupational Functioning Assessment Scale (SOFAS).

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### 5 2.4. Blood sampling and assays

Participants were instructed to have fasted (except water) since the night before for blood
sampling. They were to sit quietly and relax for 25-45 min before blood samples were obtained.
Among 1262 study participants, 1094 subjects agreed to offer blood samples in the baseline
evaluation. Serum 5-HT levels were measured using ClinRep high-performance liquid
chromatography kit (Recipe, Munich, Germany) at GreenCross LabCell (Yongin, Korea).

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### 12 **2.5. Treatment**

Details of the treatment in this study have been previously published (24). Before treatment 13 commencement, a comprehensive review was made of the patients' clinical manifestations (e.g., 14 psychotic and anxiety symptoms), severity of illness, physical comorbidities and medication 15 profiles, and history of previous treatments. Minimal and maximal dosages of pharmacological 16 17 agents were determined considering existing treatment guidelines (25, 26). In the first treatment Step 1, patients received antidepressant treatment, taking into consideration these data and 18 19 treatment guidelines (26-28), for 3 weeks. Antidepressants used were bupropion, 20 desvenlafaxine, duloxetine, escitalopram, fluoxetine, mirtazapine, paroxetine, sertraline, venlafaxine, and vortioxetine. After Step 1 antidepressant monotherapy, next step 21 pharmacotherapy could be administered every 3 weeks during the acute treatment phase (3, 6, 22 23 9, and 12 weeks with a 3-day allowable window) and every 3 months during the continuation 24 treatment phase (6, 9, and 12 months with a 7-day allowable window), whenever needed. At

the end of each step, overall effectiveness and tolerability were reviewed for proceeding with 1 2 measurement-based next-step treatments. In cases of insufficient improvement (a HAMD score 3 reduction of <30% from the baseline) or intolerable side effects, patients were instructed to choose whether they would prefer to remain in the current step or enter the next step strategies 4 5 with switching (S), augmentation (A), combination (C), S + A, S + C, A + C, and S + A + Ctreatment. Patients were also allowed to receive next step treatment if they showed sufficient 6 7 improvement (a HAMD score reduction of  $\geq$ 30% from the baseline) and absent/tolerable side 8 effects. For determining treatment strategies, each patient's preference was given priority to 9 maximize medication compliance and treatment outcomes (29). Antidepressants switched or 10 combined were bupropion, desvenlafaxine, duloxetine, escitalopram, fluoxetine, mirtazapine, paroxetine, sertraline, venlafaxine, and vortioxetine. Augmented drugs were buspiron, lithium, 11 12 triiodothyronine, and atypical antipsychotics including aripiprazole, risperidone, olanzapine, quetiapine, and ziprasidone. Since the number entered into Step 5 or above was small, treatment 13 steps were classified into Step 1, 2, 3, and 4 (including Step 5+) in the analysis. 14

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### 16 **2.6. Definition of the outcome**

17 Remission was defined as a HAMD score ≤7. Remission at 12 weeks and at 12 months was
18 used in order to investigate the associations of serum 5-HT levels with short- and long-term
19 treatment outcomes, respectively.

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### 21 2.7. Statistical analysis

Baseline data were compared by remission status in the acute treatment phase (up to 12 weeks) and continuation treatment phase (up to 12 months) using independent *T*-tests or chi-square tests. The unadjusted associations of the initially prescribed antidepressant type with 12-week

and 12-month remission were investigated using chi-square tests. Antidepressants were classified as SSRIs (escitalopram, sertraline, paroxetine, fluoxetine) and non-SSRIs, and included a noradrenergic and specific serotonin antidepressant (NaSSA; mirtazapine), serotonin-norepinephrine reuptake inhibitors (SNRIs; venlafaxine, duloxetine, desvenlafaxine), a norepinephrine-dopamine reuptake inhibitor (NDRI; bupropion), and a serotonin modulator and stimulator (vortioxetine). Serum 5-HT levels were compared by the remission status in treatment steps using independent *T*-tests. Serum 5-HT levels were compared between the four treatment steps (Step 1, 2, 3, and 4) up to 12 weeks and up to 12 months using analysis of variance with post-hoc Scheffe's tests. Moreover, serum 5-HT levels were compared by the remission status in treatment steps using independent *T*-tests.Relationships between baseline serum 5-HT levels (categorized as per 20 ng/mL increase) and 12-week and 12-month remission status were analyzed using binary logistic regression after adjustment for covariates that significantly differed (P-value < 0.05) in the baseline data, including age, monthly income, age at onset, number of depressive episodes, duration of present episode, HAMD, HADS-A, and SOFAS. All statistical tests were two-sided with a significance level of 0.05. Statistical analysis were performed using IBM SPSS Statistics (version 25). 

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### 1 **3. Results**

### 2 **3.1. Recruitment and treatment flow**

3 Patient flow by treatment steps over a 12-month period is described in Figure 1. Among 1262 patients evaluated at baseline, blood samples were obtained in 1094 (86.7%) and 1086 (86.1%) 4 5 who were followed up at least once during the 12-week treatment period. Reasons for drop-out 6 were: lack of treatment effect (N = 4) and loss to follow-up (N = 4). A total of 1086 patients, 7 included for a 12 week analysis, had lower rates of suicide attempt history but higher scores of 8 HADS-A and EQ-5D compared to 176 in the baseline. At the 12-week assessment point, 463 9 (42.6%) patients received Step 1 antidepressant monotherapy treatment, 360 (33.1%) received 10 Step 2 treatment, 200 (18.4%) received Step 3 treatment, and 63 (5.8%) received Step 4 11 treatment.

After the acute treatment phase, 884 (81.4%) were followed up at least once, up to the 12 12-month follow-up and an analysis of remission in the continuation treatment phase was 13 performed. Reasons for drop-out were: lack of treatment effect (N = 129), transfer to another 14 hospital (N = 13), intolerable side effects (N = 12), poor physical condition (N = 9), and loss 15 to follow-up (N = 39). Attrition at 12 months was significantly associated with unemployed 16 status, melancholic features, and higher EQ-5D scores at baseline. However, drop-out at 12 17 months was not associated with 5-HT level (t = 1.114, P = 0.266). At the 12-month assessment 18 point, 326 (36.9%) patients had received Step 1 treatment, 286 (32.4%) had received Step 2 19 20 treatment, 172 (19.5%) had received Step 3 treatment, and 100 (11.3%) had received Step 4 21 treatment.

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### 23 **3.2. Baseline characteristics by remission status**

24 Baseline characteristics by remission status in patients up to 12 weeks of treatment (acute

treatment phase) and up to 12 months of treatment (continuation treatment phase) are compared 1 in Table 1. Age (t = 2.610, P = 0.009), monthly income ( $\chi^2$  = 5.801, P = 0.016), age at onset (t 2 = -3.042, P = 0.002), duration of present episode (t = 3.128, P = 0.002), HAMD (t = 2.418, P = 0.002) 3 0.016), HADS-A (t = 3.666, P<0.001), and SOFAS (t = -4.785, P<0.001) were significantly 4 different between the 12-week remission group and no remission group. Monthly income ( $\chi^2$  = 5 4.465, P = 0.035), number of depressive episodes (t = 3.589, P<0.001), and HADS-A (t = 2.521, 6 7 P = 0.012) were significantly different between the 12-month remission group and no remission 8 group. There was a general pattern of better clinical presentations associated with the remission 9 group in both the acute and continuation treatment phase. The types of antidepressants initially prescribed are compared between those with and without remission in Supplementary table 2. 10 There was no difference in frequency of the types of antidepressants initially prescribed 11 between the remission and no remission groups in both acute and continuation treatment phase. 12 Considering these findings and potential collinearity between the variables, as well as previous 13 reports on remission predictors (4, 30), eight variables (age, monthly income, age at onset, 14 number of depressive episodes, duration of present episode, HAMD, HADS-A, and SOFAS) 15 were included as covariates in the later adjusted analysis. 16

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### 18 **3.3. Baseline serum 5-HT levels by treatment steps**

Baseline serum 5-HT levels by treatment steps are compared in Supplementary Table 1. Baseline serum 5-HT levels were significantly different between treatment steps in both the acute treatment phase (F = 5.597, P = 0.001) and continuation treatment phase (F = 5.176, P = 0.002). In *post-hoc* comparisons, patients who received Step 1 treatment showed higher baseline serum 5-HT levels compared to patients who received Step 3 or Step 4 treatment in the acute treatment phase. Similar to the acute treatment phase, patients who received Step 1 or Step 2 treatment showed higher baseline serum 5-HT levels than patients who received Step
4 treatment in the continuation treatment phase. Taken together, baseline serum 5-HT levels
were higher in the lower step treatment regardless of the treatment period.

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### 5 3.4. Baseline serum 5-HT levels by remission status and treatment steps

6 Baseline serum 5-HT levels by 12-week and 12-month remission and treatment steps are 7 compared in Table 2. Up to 12 weeks of treatment, the remission group showed higher baseline serum 5-HT levels compared with the no remission group (t = -2.083, P = 0.037). Among 8 9 treatment steps in the acute treatment phase, not in patients who received Step 2 (t = -1.129, P = 0.259), Step 3 (t = -0.812, P = 0.418), and Step 4 (t = -0.762, P = 0.449), but among those in 10 Step 1 (t = -2.276, P = 0.023) treatment, higher baseline serum 5-HT levels in the remission 11 group compared to the no remission group were shown. Up to 12 months of treatment, there 12 was no difference of baseline serum 5-HT levels between the remission group and no remission 13 group (t = -1.216, P = 0.224). However, in the patients who received Step 1 treatment, baseline 14 serum 5-HT levels were higher in the remission group compared to the no remission group (t 15 = -2.330, P = 0.020). These data indicate that baseline serum 5-HT levels of the remission group 16 17 are higher than those of the no remission group in patients who received Step 1 treatment, regardless of the treatment period. 18

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# 3.5. Adjusted associations of baseline serum 5-HT levels with 12 week and 12 month remission

Unadjusted and adjusted associations of baseline serum 5-HT levels with 12-week and 12month remission by treatment steps are summarized in Table 3. Baseline serum 5-HT levels showed positive associations with 12-week remission. Adjusted logistic regression analysis,

1	after adjustment for seven variables mentioned above, showed that high baseline serum 5-HT
2	levels are significantly associated with 12-week remission (OR = $1.061$ , P = $0.015$ ). Among
3	treatment steps, not in patients who received Step 2 (OR = $1.040$ , P = $0.355$ ), Step 3 (OR = $1.040$
4	1.065, P = 0.298), and Step 4 (OR = $1.095, P = 0.378$ ), but among those in Step 1 (OR= $1.061, P = 0.298$ )
5	P=0.015) treatment, positive associations between baseline serum 5-HT levels and 12-week
6	remission were shown. Unlike the 12-week remission, there was no association between 12-
7	month remission and baseline serum 5-HT levels ( $OR = 1.039$ , $P = 0.200$ ). However, patients
8	who received Step 1 treatment showed positive associations between baseline serum 5-HT
9	levels and 12-month remission in both unadjusted (OR = $1.130$ , P = $0.014$ ) and adjusted (OR
10	= 1.133, $P = 0.019$ ) logistic regression analysis. These data demonstrate that baseline serum 5-
11	HT levels are positively associated with 12-week and 12-month remission in patients who
12	received Step 1 treatment.
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### 1 **4. Discussion**

In the present study, by using data from a naturalistic prospective study, which reflects realworld clinical practice, we determined that baseline serum 5-HT levels were higher in the remission group, and that high baseline serum 5-HT levels are associated with 12-week and 12-month remission in patients with depressive disorders who are treated with antidepressant monotherapy. In addition, baseline serum 5-HT levels were higher in lower treatment steps regardless of treatment period.

8 Results from the acute treatment phase, particularly in patients who received Step 1 treatment, showed that baseline serum 5-HT levels were higher in the remission group and high 9 10 baseline serum 5-HT levels were associated with 12-week remission. These results are similar 11 to previous studies, which observed that baseline plasma 5-HT levels are higher in responders, and that high baseline plasma 5-HT levels are associated with 4- and 8-week outcomes in 12 patients who received SSRI monotherapy (15, 16). Going one step further than previous studies, 13 14 we demonstrated the utility of baseline 5-HT levels as a biomarker for treatment outcomes not only in patients treated with SSRIs, but also in those treated with other types of antidepressants, 15 16 such as NaSSAs, SNRIs, NDRIs, and serotonin modulators and stimulators. Since various types of antidepressants are used as initial treatment for depressive disorders in real-world 17 18 clinical practice, our results shed light on using baseline 5-HT levels as a biomarker for 19 predicting treatment outcomes in patients receiving antidepressant monotherapy. Although our 20 findings are novel, care should be taken in their interpretation because type of antidepressant was not controlled for and there were many drop-outs during the continuation treatment phase. 21 22 Further large-scale randomized studies are needed to confirm the results.

In this study, we have newly discovered that high baseline serum 5-HT levels are associated not only with short-term outcomes but also with long-term outcomes in patients who receive Step 1 treatment. Although there were no significant differences of baseline serum 5-HT levels between the remission group and no remission group among all participants in the continuation phase, the remission group had higher baseline serum 5-HT levels in the Step 1 treatment subgroup. Furthermore, high baseline serum 5-HT levels were associated with 12month remission in patients who received Step 1 treatment. These findings suggest that baseline serum 5-HT levels might be used as a biomarker for predicting long-term outcomes as well as short-term outcomes in patients who receive antidepressant monotherapy.

8 Based on our results, baseline serum 5-HT levels seem to lose effectiveness as a 9 biomarker for predicting treatment outcomes as the treatment strategy becomes complicated in depressive disorders. Among the treatment steps, only Step 1 treatment showed associations 10 between high baseline serum 5-HT levels and remission in both the acute and continuation 11 12 treatment phase. In addition, patients who were treated by simple strategies had higher baseline serum 5-HT levels compared to patients treated by complicated strategies in both acute and 13 14 continuation treatment phases. From these results, we speculate that baseline serum 5-HT level can function as a biomarker for treatment outcomes only when it is sufficiently high. However, 15 further investigations are needed to clarify this idea. 16

17 Several issues should be borne in mind before drawing a conclusion. First, central and peripheral 5-HT systems are functionally separated and correlation between peripheral blood 18 19 and cerebrospinal fluid 5-HT levels are inconclusive (31, 32). However, results from our study 20 and others (15, 16) show that baseline peripheral blood 5-HT levels are associated with treatment outcomes in patients who receive antidepressant monotherapy. Further studies are 21 22 needed to uncover the detailed mechanisms of how high peripheral blood 5-HT levels are 23 connected with better antidepressant outcomes. Second, we measured 5-HT in serum rather 24 than plasma. In the periphery, most of 5-HT is synthesized by enterochromaffin cells in the gut

(33, 34). Once released from the gut, most 5-HT is taken up into platelets (>95%) by 5-HT 1 2 transporter, with the remaining free 5-HT levels very low in circulation (35-37). While plasma 5-HT levels reflect only the bioactive free 5-HT in the peripheral blood, serum 5-HT levels 3 reflect some portion of platelet 5-HT (38). Our study showed that, as well as plasma 5-HT 4 5 levels, serum 5-HT levels could also be used as a biomarker for antidepressant treatment 6 outcomes. From these results, we hypothesize that both the bioactive 5-HT and 5-HT pool in 7 the periphery might predict the antidepressant treatment outcomes in patients with depressive 8 disorders. To verify this hypothesis further, it would be of interest to measure platelet 5-HT 9 levels in patients who receive antidepressant treatment with depressive disorders.

10 There are several limitations to this study. First, unlike previous studies, which showed that a greater decrease in plasma 5-HT levels as well as higher baseline plasma 5-HT levels are 11 12 associated with better clinical outcomes (15, 16), we were unable to determine whether treatment-related changes in serum 5-HT levels are associated with treatment outcomes, 13 because we did not obtain serum 5-HT levels during the follow-up period. However, since a 14 greater decrease in plasma 5-HT levels was largely attributed to higher baseline plasma 5-HT 15 levels, rather than to different follow-up period plasma 5-HT levels between responders and 16 17 non-responders in one previous study (16), we speculate that our study would have shown similar results if the follow-up period serum 5-HT levels had been measured. Second, as this 18 19 study used a naturalistic prospective design, treatment was in accordance with patient 20 preference under a clinician's guidance rather than being determined by a predetermined protocol. Third, follow-up rates were reduced in the continuation treatment phase compared to 21 22 the acute treatment phase. Study participants who were lost to follow-up had poor prognostic 23 characteristics at baseline such as unemployed status, melancholic features, and higher EQ-5D 24 scores, which might have obscured the results. Fourth, since various antidepressants were

initially used and different treatment strategies (switching, augmentation, combination, and
mixtures of these approaches) were applied from 3 weeks after the start of antidepressant
monotherapy, there were too many variables to evaluate the effect of 5-HT on antidepressant
response by the type of antidepressant.

5 This study has several strengths. It was a naturalistic prospective study; therefore, 6 results reflect actual clinical practice situations. We followed up patients up to 12 months to 7 evaluate the long-term outcomes of treatment. Moreover, since we assessed various factors 8 associated with treatment outcomes in the baseline, including socio-demographic 9 characteristics, clinical characteristics, and assessment scales, we verified the independent role 10 of baseline serum 5-HT levels as a biomarker for short- and long-term treatment outcomes in 11 depressive disorders, considering multiple confounders.

12 Although antidepressants are the first-line treatment for moderate to severe depressive disorders, less than one third of patients achieve remission after the first trial. However, proper 13 14 biomarkers predicting outcomes before starting antidepressant treatments are lacking. This study verified that baseline serum 5-HT levels are higher in the remission group, and high 15 baseline serum 5-HT levels are associated with remission in acute and continuation treatment 16 17 phases in patients who receive antidepressant monotherapy. By these results, we suggest that baseline serum 5-HT levels might be a biomarker for predicting treatment outcomes in patients 18 19 who receive antidepressant monotherapy. Further studies should evaluate whether treatment-20 related changes of serum 5-HT levels are associated with short- and long-term outcomes. As this study was a non-randomized trial, effectiveness of baseline serum 5-HT levels as a 21 biomarker for predicting treatment outcomes in patients who receive antidepressants should be 22 23 evaluated in randomized trials in the future.

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- 2 None.
- 3

### **4 Declaration of Competing Interest**

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### 10 **CRediT authorship contribution statement**

Wonsuk Choi: Conceptualization, Data curation, Formal analysis, Writing. Hee-Ju Kang: 11 Data curation, Methodology. Ju-Wan Kim: Formal analysis, Methodology. Hee Kyoung Kim: 12 13 Data curation, Validation, Project administration. Ho-Cheol Kang: Data curation, Validation, 14 Project administration. Ju-Yeon Lee: Data curation, Validation, Project administration. Sung-Kim: Data curation, Validation, Project administration. **Robert** Stewart: 15 Wan Conceptualization, Formal analysis, Writing. Jae-Min Kim: Conceptualization, Data curation, 16 Formal analysis, Writing 17

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### **19 Statement of Ethics**

All patients gave written informed consent to participate in the study and use their data. The study was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2008 and approved by the Ethics Commission of the Chonnam National University Hospital Institutional Review Board (CNUH 2012-014) as it uses de-identified data. It was registered at
 cris.nih.go.kr (identifier: KCT0001332).

3

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9 Health Research (NIHR) Senior Investigator.

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# Table 1

Baseline characteristics by 12-week and 12-month remission in blood obtained from patients with depressive disorders

	Up	to 12-week tro	eatment (N = 108	86)	Up	Up to 12-month treatment (N = 884)			
	No remission (N = 596)	Remission (N = 490)	Statistical coefficients <sup>a</sup>	P-value	No remission (N = 259)	Remission (N = 625)	Statistical coefficients <sup>a</sup>	P-value	
Socio-demographic characteristics									
Age, mean (SD) years	55.9 (15.6)	58.2 (13.9)	t = 2.610	P = 0.009	56.1 (16.0)	57.3 (14.2)	t = -1.048	P = 0.295	
Gender, N (%) female	411 (69.0)	334 (68.2)	$\chi^2 = 0.079$	P = 0.778	173 (66.8)	434 (69.4)	$\chi^2 = 0.595$	P = 0.440	
Education, mean (SD) years	9.1 (4.7)	9.1 (4.9)	t = -0.065	P = 0.948	9.3 (4.7)	9.0 (4.8)	t = 1.090	P = 0.276	
Marital status, N (%) unmarried	192 (32.2)	134 (27.3)	$\chi^2 = 3.033$	P = 0.082	83 (32.0)	174 (27.8)	$\chi^2 = 1.571$	P = 0.210	
Living alone, N (%)	94 (15.8)	73 (14.9)	$\chi^2 = 0.158$	P = 0.691	43 (16.6)	88 (14.1)	$\chi^2 = 0.923$	P = 0.337	
Religious observance, N (%)	326 (54.7)	281 (57.3)	$\chi^2 = 0.765$	P = 0.382	141 (54.4)	355 (56.8)	$\chi^2 = 0.414$	P = 0.520	
Unemployed status, N (%)	186 (31.2)	130 (26.5)	$\chi^2 = 2.852$	P = 0.091	82 (31.7)	160 (25.6)	$\chi^2 = 3.383$	P = 0.066	
Monthly income, N (%) <2,000 USD	375 (62.9)	273 (55.7)	$\chi^2 = 5.801$	P = 0.016	167 (64.5)	355 (56.8)	$\chi^2 = 4.465$	P = 0.035	
Clinical characteristics									
Major depressive disorder, N (%)	510 (85.6)	415 (84.7)	$\chi^2 = 0.164$	P = 0.686	227 (87.6)	534 (85.4)	$\chi^2 = 0.743$	P = 0.389	
Melancholic feature, N (%)	95 (15.9)	67 (13.7)	$\chi^2 = 1.088$	P = 0.297	45 (17.4)	96 (15.4)	$\chi^2 = 0.554$	P = 0.457	
Atypical feature, N (%)	35 (5.9)	34 (6.9)	$\chi^2 = 0.514$	P = 0.473	16 (6.2)	39 (6.2)	$\chi^2 = 0.001$	P = 0.972	
Age at onset, mean (SD) years	50.5 (17.3)	53.6 (15.7)	t = -3.042	P = 0.002	50.6 (17.7)	52.3 (16.1)	t = -1.328	P = 0.185	

Duration of illness, mean (SD) years	5.4 (9.3)	4.7 (8.7)	t = 1.311	P = 0.190	5.5 (9.0)	5.0 (9.1)	t = 0.739	P = 0.460
Recurrent depression, N (%)	322 (54.0)	248 (50.6)	$\chi^2 = 1.257$	P = 0.262	145 (56.0)	321 (51.4)	$\chi^2 = 1.571$	P = 0.210
Number of depressive episodes, mean (SD)	1.2 (1.5)	1.0 (1.4)	t = 1.836	P = 0.067	1.4 (1.8)	1.0 (1.3)	t = 3.589	P < 0.001
Duration of present episode, mean (SD) months	8.3 (12.0)	6.4 (8.0)	t = 3.128	P = 0.002	8.3 (12.2)	7.3 (9.5)	t = 1.138	P = 0.256
Family history of depression, N (%)	82 (13.8)	76 (15.5)	$\chi^2 = 0.664$	P = 0.415	32 (12.4)	93 (14.9)	$\chi^2 = 0.961$	P = 0.327
History of suicide attempt, N (%)	56 (9.4)	39 (8.0)	$\chi^2 = 0.695$	P = 0.404	27 (10.4)	45 (7.2)	$\chi^2 = 2.545$	P = 0.111
Number of physical disorders, mean (SD)	1.6 (1.3)	1.7 (1.2)	t = -1.212	P = 0.226	1.7 (1.3)	1.7 (1.3)	t = 0.021	P = 0.983
Assessment scales, mean (SD) scores								
Hamilton Depression Rating Scale	21.0 (4.1)	20.4 (4.1)	t = 2.418	P = 0.016	20.8 (4.2)	20.1 (4.1)	t = 0.427	P = 0.669
Hospital Anxiety & Depression Scale-anxiety subscale	12.2 (4.0)	11.3 (4.1)	t = 3.666	P < 0.001	12.3 (4.0)	11.6 (4.0)	t = 2.521	P = 0.012
EuroQol-5D	9.0 (1.6)	8.8 (1.4)	t = 1.767	P = 0.078	9.0 (1.5)	8.8 (1.5)	t = 1.607	P = 0.108
Social and Occupational Functional Assessment Scale	55.0 (7.6)	57.1 (7.1)	t = -4.785	P < 0.001	55.6 (7.0)	56.3 (7.3)	t = -1.028	P = 0.227

<sup>a</sup>Independent two sample t-test or  $\chi^2$  tests, as appropriate.

# Table 2

	N	No remission		Remission	Statistical coefficients <sup>a</sup>	P-value	
	N	Mean (SD)	Ν	Mean (SD)			
Up to 12-week treat	ment						
All participants	596	76.8 (50.2)	490	83.4 (50.2)	t = -2.083	0.037	
Step 1	291	81.2 (50.6)	172	92.4 (52.6)	t = -2.276	0.023	
Step 2	184	77.8 (50.1)	176	83.5 (52.8)	t = -1.129	0.259	
Step 3	94	67.7 (45.8)	106	73.6 (56.5)	t = -0.812	0.418	
Step 4	27	57.2 (54.0)	36	69.4 (68.6)	t = -0.762	0.449	
Up to 12-month trea	tment						
All participants	259	75.6 (51.7)	625	80.3 (52.3)	t = -1.216	0.224	
Step 1	112	75.6 (50.6)	214	89.1 (49.4)	t = -2.330	0.020	
Step 2	83	78.7 (48.5)	203	82.0 (51.8)	t = -0.486	0.627	
Step 3	32	82.0 (61.4)	140	73.0 (51.6)	t = 0.774	0.443	
Step 4	32	61.4 (53.1)	68	62.9 (58.6)	t = -0.119	0.906	

Baseline serum 5-HT levels by 12-week and 12-month remission and treatment steps

<sup>a</sup>Independent two sample t-test.

### Table 3

Associations	of baselin	e serum 5-HT	levels with	12-week and	112-month	remission
1 100000100000		• • • • • • • • • • • • • • • • • • • •				

	Unadjusted OR (95% CI)	P-value	Adjusted OR (95% CI)	P-value
12-week remission				
All participants (N = 1086)	1.050 (1.004-1.099)	0.034	1.061 (1.012-1.112)	0.015
Step 1 (N = 463)	1.097 (1.020-1.180)	0.013	1.135 (1.048-1.230)	0.002
Step 2 (N = 360)	1.041 (0.961-1.128)	0.320	1.040 (0.957-1.131)	0.355
Step 3 (N = 200)	1.037 (0.930-1.155)	0.516	1.065 (0.946-1.198)	0.298
Step 4 (N = 63)	1.073 (0.909-1.266)	0.403	1.095 (0.895-1.339)	0.378
12-month remission				
All participants (N = 884)	1.033 (0.976-1.093)	0.260	1.039 (0.980-1.101)	0.200
Step 1 (N = 326)	1.130 (1.025-1.246)	0.014	1.133 (1.021-1.258)	0.019
Step 2 (N = 286)	1.011 (0.915-1.118)	0.828	1.019 (0.918-1.132)	0.719
Step 3 (N = 172)	0.929 (0.808-1.067)	0.297	0.931 (0.799-1.084)	0.358
Step 4 (N = 100)	1.018 (0.877-1.182)	0.815	1.034 (0.870-1.228)	0.705

Adjusted odds ratio (OR) after adjustment for age, monthly income, age at onset, number of depressive episodes, duration of present episode, Hamilton Depression Rating Scale, Hospital Anxiety and Depression Scale-anxiety subscale, and Social and Occupational Functional Assessment Scale.

# Supplementary Table 1

Baseline serum 5-HT levels by treatment steps

	Mean (SD)	F	P-value	Post hoc tests
Up to 12-week treatment (N = 1086)	79.8 (52.6)			
Step 1 (N = 463)	85.3 (51.6)	5.597	0.001	Step 1>3,4
Step 2 (N = 360)	80.4 (51.5)			
Step 3 (N = 200)	70.8 (51.7)			
Step 4 (N = $63$ )	64.1 (62.6)			
Up to 12-month treatment (N = 884)	78.9 (52.1)			
Step 1 (N = 326)	84.4 (50.2)	5.176	0.002	Step 1,2>4
Step 2 (N = 286)	81.0 (50.8)			
Step 3 (N = 172)	74.6 (53.5)			
Step 4 (N = 100)	62.4 (56.6)			

### **Supplementary Table 2.**

#### Type of antidepressant, N (%) Up to 12-week treatment Up to 12-month treatment No remission Remission Statistical P-value No remission Remission Statistical (N = 596)coefficients (N = 259)(N = 490)(N = 625)coefficients **SSRIs**<sup>a</sup> $\chi^2 = 2.234$ $\chi^2 = 1.648$ 358 (60.1) 316 (64.5) 0.135 156 (60.2) 405 (64.8) Non-SSRIs<sup>b</sup> 238 (39.9) 174 (35.5) 103 (39.8) 220 (35.2)

P-value

0.199

Initially prescribed antidepressant types by remission status

SSRI, selective serotonin reuptake inhibitor.

<sup>a</sup>Escitalopram, sertraline, paroxetine, and fluoxetine were included as SSRIs.

<sup>b</sup>Mirtazapine, venlafaxine, duloxetine, desvenlafaxine, bupropion, and vortioxetine were included as non-SSRIs.



Fig. 1. Participants flow