



## Correlations between serum cetuximab and EGFR-related markers, and skin disorders in head and neck cancer patients

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Original Article

Correlations between serum cetuximab and EGFR-related markers, and skin disorders in head  
and neck cancer patients

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## **Abstract**

**Purpose:** Cetuximab inhibits epidermal growth factor receptor (EGFR) signaling in cancer and skin cells, thereby inducing anti-cancer effects and skin disorders. The present study aimed to evaluate the relationships between serum cetuximab and EGFR-related markers, and adverse effects in head and neck cancer patients.

**Methods:** Thirty-four head and neck cancer patients receiving weekly intravenous cetuximab were enrolled. Serum cetuximab levels were determined just before dosing. Blood samples for determination of serum EGFR-related markers including soluble epidermal growth factor receptor (sEGFR) and interleukin-6 (IL-6) were obtained. The severities of skin disorders, their medications, and hypomagnesemia treatment were also assessed.

**Results:** Serum levels of cetuximab and sEGFR were negatively and positively correlated with that of IL-6, respectively. The serum cetuximab level was 2-fold higher in the patients with a grade 2–3 skin rash than with a grade 0–1 rash. The serum cetuximab cut-off value related to severe skin rash was 71 µg/mL (sensitivity, 59%; and specificity, 94%). The use of a strong topical corticosteroid for skin rash was also associated with a higher serum cetuximab level. Serum levels of sEGFR and IL-6 had no correlations with the skin disorder severities or their medications. Hypomagnesemia treatment using intravenous magnesium sulfate was not related to serum cetuximab and EGFR-related markers.

**Conclusions:** Head and neck cancer patients with a higher serum IL-6 level tended to have a lower serum cetuximab level. Serum cetuximab had positive correlations to skin rash severity

and its medication in the study population.

**Key words:**

cetuximab; pharmacokinetics; epidermal growth factor receptor; interleukin-6; skin disorder; head and neck cancer

**Abbreviations:**

EGFR, epidermal growth factor receptor; sEGFR, soluble EGFR; STAT3, signal transducer and activator of transcription 3; JAK2, Janus kinase 2; IL-6, interleukin-6; CRP, C-reactive protein; LC-MS/MS, liquid chromatography system coupled to a tandem mass spectrometry; ELISA, enzyme-linked immunosorbent assay; ROC, Receiver operating characteristic; OR, odds ratios; 95% CI, 95% confidence intervals; IQR, interquartile range; AUC, area under the curve

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## **Introduction**

Cetuximab, an anti-epidermal growth factor receptor (EGFR) monoclonal antibody, is commonly used for the treatment of solid cancers such as head and neck cancer, and colorectal cancer [1–3]. The EGFR overexpression observed in these cancer cells is closely related to cancer progression and metastasis [4,5]. Ligand binding to EGFR activates its intracellular signal cascades and promotes the synthesis of various proteins in cancer cells. In cancer patients, the EGFR activation influences blood circulating levels of endogenous markers such as soluble receptors and cytokines [6–9]. Cetuximab binds to EGFR with high affinity and inhibits its downstream signaling [10,11]. Few clinical reports on the relationships between serum cetuximab and endogenous markers have been published.

The EGFR is proteolytically cleaved by cell surface proteins and constitutively releases soluble EGFR (sEGFR). The sEGFR with its high affinity for EGFR ligands interferes with ligand-dependent EGFR activation. The sEGFR regulates cancer progression by inhibiting EGFR signal transduction [12–14]. Serum sEGFR is used as a diagnostic marker for several cancers [6,15–17]. In cervical cancer patients, the serum sEGFR level was higher than in healthy adults and was elevated according to the disease progression [18]. The increased serum sEGFR was associated with good overall survival in metastatic breast cancer patients receiving chemotherapy [19]. Cetuximab has been shown to reduce proteolytic sEGFR release by interfering with interactions of its shedding regulators with EGFR [20,21]. The serum kinetic relationship between sEGFR and cetuximab remains unclear in cancer patients.

In head and neck squamous cell carcinomas, persistent EGFR stimulation leads to constitutive activation of the signal transducer and activator of transcription 3 (STAT3) [22]. STAT3 is also activated by Janus kinase 2 (JAK2) on interleukin-6 (IL-6) receptors in an autocrine/paracrine manner [23–25]. In ovarian cancer cells, EGFR activation has been found to enhance IL-6 production through the cross-talk of downstream JAK2/STAT3 signals [25]. Cetuximab blocks the downstream signal pathways of EGFR and thereby inhibits the JAK2/STAT3 signaling [11,26]. Therefore, cetuximab may affect serum IL-6 levels in head and neck cancer patients. Serum IL-6 has been shown to be a candidate prognostic marker for cetuximab-containing therapies [27]. The association between serum levels of cetuximab and IL-6 needs to be characterized.

Cancer patients treated with intravenous cetuximab frequently exhibit skin disorders that affect continuation of treatment and its associated prolongation of overall survival [1,2,28]. Cetuximab inhibits EGFR in keratinocytes and induces impairment of skin barriers and regeneration [28]. In contrast, the severity of skin rash has been reported to be positively associated with the cetuximab-related anti-cancer effect [29,30]. The anti-cancer effect of cetuximab can be partially explained by its pharmacokinetics [31]. The IL-6 on keratinocytes is involved in epidermal barrier repair and is upregulated by EGFR inhibitor [32]. Serum sEGFR was decreased in patients with autoimmune skin disease than without [33]. The serum cetuximab and EGFR-related markers may have associations with the severities of skin disorders. Additionally, the inhibition of EGFR by cetuximab induces hypomagnesemia as a

critical adverse event [34]. Clinical factors associated with a decrease in serum magnesium need to be clarified.

Serum markers related to pharmacokinetics and adverse effects of cetuximab should be investigated in order to optimize the safety and efficacy of cetuximab therapy in cancer patients. The present study aimed to evaluate the correlations between serum cetuximab and EGFR-related markers, and adverse effects in head and neck cancer patients.

## **Materials and methods**

### **Patients and study schedule**

This prospective observational study was conducted at Hamamatsu University Hospital (Hamamatsu, Japan). Japanese head and neck cancer patients who received intravenous cetuximab (Erbix Injection, Merck Serono, Tokyo, Japan) weekly at a loading dose of 400 mg/m<sup>2</sup> followed by a maintenance dose of 250 mg/m<sup>2</sup> were recruited. The bivariate correlation analysis using an effect size of 0.5, a statistical power of 0.8, and a significance level of 0.05 needed a total sample number of 29. The target number of recruited patients was set to 40 in order to analyze the serum markers correlations and consider the patient exclusion. All patients were fully informed of the scientific aim of this study and each patient provided written informed consent before study enrollment. As a result, we enrolled 34 cancer patients receiving intravenous cetuximab according to the exclusion criteria, which were as follows: (1) patients who discontinued or postponed their cetuximab treatment in week 1–4, (2) patients who were

being co-treated with potent IL-6 suppressants such as calcineurin inhibitors, JAK inhibitors, anti-IL-6 receptor antibody drugs, or anti-tumor necrosis factor- $\alpha$  antibody drugs; (3) patients who were being daily treated with potent topical corticosteroids before the first cetuximab administration; (4) patients who were concomitantly receiving medications for atopic dermatitis; (5) patients who had an active autoimmune disease with serum C-reactive protein (CRP) > 2.0 mg/dL; and (6) patients who suffered from an active infection with body temperature > 38°C during the study period. Blood specimens were collected from a forearm vein into tubes just before dosing at week 4 or later. Eleven patients sequentially donated serum specimens before the first cetuximab administration and at week 2, 3, 4, 5, and/or later. This study is registered with the University Hospital Medical Information Network (UMIN000027016).

### **Determination of serum cetuximab**

Serum cetuximab levels were determined by a validated liquid chromatography system coupled to a tandem mass spectrometry (LC-MS/MS) method with immobilized tryptic digestion [35]. The calibration curve of serum cetuximab ranges from 4 to 200  $\mu\text{g}/\text{mL}$  ( $r = 0.998$ ). The lower limit of quantification of serum cetuximab was 4  $\mu\text{g}/\text{mL}$ . The intra- and inter-assay accuracies and imprecisions of serum cetuximab were 88.0–100.7% and less than 13.2%, respectively. The serum cetuximab levels in the LC-MS/MS method were correlated with those in commercially available enzyme-linked immunosorbent assay (ELISA) ( $r = 0.90$ ,  $P < 0.01$ ) and the mean bias

was 1.5% in head and neck cancer patients.

### **Determination of serum sEGFR and IL-6**

The serum sEGFR level was determined using a commercially available ELISA kit from Abcam plc. (Cambridge, UK). The calibration curve range was 78–5,000 pg/mL for serum sEGFR and its detection limit was 1 pg/mL. The intra- and inter-assay imprecisions of serum sEGFR were both < 3.0%. The serum IL-6 level was measured using a commercially available ELISA kit from BioLegend Inc. (San Diego, CA, USA). The calibration curve range for serum IL-6 was 7.8–500 pg/mL and its detection limit was 1.6 pg/mL. The intra- and inter-assay accuracies and imprecisions of serum IL-6 were 93.9–102.6% and less than 13.0%, respectively.

### **Investigation of skin disorders and skin treatments**

Skin disorders including skin rash, dry skin, and paronychia were diagnosed by one dermatologist who was blinded to the serum levels of cetuximab, sEGFR, and IL-6 within 2 days before and after blood sampling. The severities of any skin disorders were assessed using the grading system created by the National Hospital Organization Shikoku Cancer Centers in Japan (Table S1). These criteria are a modified version of the CTCAE ver. 4.0 and focus on subjective symptoms in patients. The study dermatologist managed the skin disorders according to the guideline that provides suitable treatments corresponding to each skin disorder severity in the above evaluating system [36]. Skin rashes on the face, extremity, trunk, and scalp were

treated with a topical corticosteroid and/or an oral antibiotic. The potencies of the topical corticosteroids including ointments, creams, and lotions were classified into 5 groups: weak (prednisolone), medium (hydrocortisone acetate, prednisolone valerate acetate, alclometasone dipropionate), strong (betamethasone valerate), very strong (betamethasone butyrate propionate, diflucortolone valerate, difluprednate), and strongest (clobetasol propionate). Minocycline hydrochloride capsules were orally administered at 50–100 mg 1–2 times a day. Dry skin was treated with moisturizing drugs and skin care products. Nails with paronychia were protected with fludrocortide tape and treated with topical corticosteroids and adapalene gel.

### **Investigation of hypomagnesemia and its treatment**

The patients with hypomagnesemia ( $< 1.4$  mg/dL) received magnesium supplementation. The intravenous infusion of magnesium sulfate (20 mEq elemental magnesium) was used for hypomagnesemia treatment. The serum magnesium level and hypomagnesemia treatment within 4 weeks after blood sampling for serum cetuximab were investigated by medical records.

### **Statistical analysis**

The priori analysis to estimate a sample size needed for the study and post hoc analysis for a statistical power were performed by G\*Power 3.1 software (Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany). The other statistics were calculated using IBM SPSS 25

software (IBM Japan Ltd, Tokyo). Differences in serum levels of cetuximab and EGFR-related markers between administration weeks were evaluated using the Kruskal-Wallis test, followed by the Mann-Whitney *U* test with the Bonferroni correction. The correlations between serum levels of cetuximab and EGFR-related markers were evaluated using the Pearson correlation coefficient test. The associations between severities of skin disorders or their skin treatments and serum levels of cetuximab or EGFR-related markers were analyzed using the Mann-Whitney *U* test. Receiver operating characteristic (ROC) curves were analyzed to determine the cut-off value at which the serum cetuximab level had optimum sensitivity and specificity to associate with a grade 2–3 skin rash and its skin treatment using stronger topical corticosteroid. Logistic regression analysis was used to obtain odds ratios (OR) between serum cetuximab level as the independent variable and severe skin rash or co-treatment of stronger topical corticosteroid as the dependent variables. The OR and their 95% confidence intervals (95% CI) were calculated. Differences in serum levels of cetuximab and EGFR-related markers between patients with and without hypomagnesemia treatment were analyzed using the Mann-Whitney *U* test. In the patients not receiving hypomagnesemia treatment, the correlations of serum magnesium level with serum levels of cetuximab and EGFR-related markers were evaluated using the Pearson correlation coefficient test. All values are expressed as the median and interquartile range (IQR) unless otherwise stated. A  $P < 0.05$  was considered to indicate statistical significance.

## **Results**

### **Patient characteristics**

Table 1 shows the patient characteristics of this study population. The patients were diagnosed as cancer stage III (n = 1) or IV (n = 33). Eighteen patients had distant metastases to bone, lung, and/or liver. The patients either received cetuximab monotherapy (n = 10) or concomitant cetuximab therapy with intravenous cisplatin/5-fluorouracil therapy (n = 7), intravenous paclitaxel therapy (n = 11), or radiation therapy (n = 6). The patients had low serum levels of total protein (median, 6.3 g/dL) and albumin (3.6 g/dL). The median serum CRP level was 0.81 (IQR, 0.27–2.34) mg/dL.

### **Time courses of serum cetuximab and EGFR-related markers**

Figure 1 shows the serum level-time profiles for cetuximab, sEGFR, and IL-6 in 11 patients who donated sequential serum specimens. No difference was observed in serum cetuximab levels between administration weeks 2–6 ( $P = 0.796$ ). In contrast, there was a significant difference in serum sEGFR levels between weeks 1–6 ( $P = 0.019$ ), and serum sEGFR only at week 2 was significantly lower than that at week 1 ( $P = 0.013$ ). There was no difference in serum IL-6 levels between weeks 1–6 ( $P = 0.927$ ).

### **Correlations between serum cetuximab and EGFR-related markers**

The median serum level of cetuximab at week 4 or later was 50.7  $\mu\text{g/mL}$  in the patients. A large

interindividual variation was observed in serum cetuximab level (IQR, 34.3–83.2 µg/mL). The median serum levels of sEGFR and IL-6 were 25.7 (IQR, 20.5–29.4) ng/mL and 25.1 (IQR, 18.7–47.0) pg/mL, respectively. The serum levels of cetuximab and sEGFR had a negative correlation ( $R^2 = 0.116$ ,  $P = 0.049$ ) and positive correlation ( $R^2 = 0.118$ ,  $P = 0.047$ ) with that of IL-6, respectively (Fig. 2). In contrast, the serum cetuximab level was not correlated with that of sEGFR. The serum level of CRP was positively correlated with that of sEGFR ( $R^2 = 0.120$ ,  $P = 0.044$ ) and IL-6 ( $R^2 = 0.445$ ,  $P < 0.001$ ), but not cetuximab ( $R^2 = 0.091$ ,  $P = 0.083$ ) (Fig. S1).

### **Relationships with skin disorders**

The numbers of patients with skin rash of grade 0, 1, 2, and 3 were 5, 12, 16, and 1, respectively. The serum cetuximab level was significantly higher in the patients with a grade 2–3 skin rash than with grade 0–1 ( $P = 0.013$ ) (Fig. 3). In contrast, the severity of skin rash was not associated with the serum levels of sEGFR ( $P = 0.586$ ) and IL-6 ( $P = 0.205$ ). In the ROC curve of serum cetuximab related to a grade 2–3 skin rash, the area under the curve (AUC) (0.75, 95% CI: 0.57–0.92) was significantly higher than 0.50 ( $P = 0.014$ ). The cut-off value of serum cetuximab was 71.0 µg/mL in the curve and its sensitivity and specificity were 58.8% and 94.1%, respectively. The risk of a grade 2–3 skin rash showed 1.04-fold increase accompanying with 1 µg/mL elevation of serum cetuximab (OR = 1.04, 95% CI: 1.01–1.06,  $P = 0.016$ ). The severity of the dry skin was not associated with the serum levels of cetuximab ( $P = 0.514$ ), sEGFR ( $P$

= 0.878), and IL-6 ( $P = 0.645$ ) (Fig. S2). The severity of paronychia was not related to the serum levels of cetuximab ( $P = 0.926$ ), sEGFR ( $P = 0.669$ ), and IL-6 ( $P = 0.304$ ).

### **Relationships with skin treatments**

The potency of topical corticosteroids used for skin rash in the patients was medium ( $n = 13$ ), strong ( $n = 1$ ), very strong ( $n = 13$ ), or strongest ( $n = 1$ ). Six patients were nonusers of topical corticosteroids. The serum cetuximab level was significantly higher in the patients co-treated with the stronger topical corticosteroids than with the medium topical corticosteroids or nonusers ( $P = 0.001$ ) (Fig. 4). In contrast, topical corticosteroid potency was not associated with the serum levels of sEGFR ( $P = 0.167$ ) and IL-6 ( $P = 0.066$ ). In the ROC curve of serum cetuximab related to skin rash treatment using stronger topical corticosteroid, the AUC (0.84, 95% CI: 0.68–0.99) was significantly higher than 0.50 ( $P = 0.001$ ). The cut-off value of serum cetuximab was 76.0  $\mu\text{g/mL}$  in the curve and its sensitivity and specificity were 66.7% and 100%, respectively. The OR for co-treatment of stronger topical corticosteroid in relation to serum cetuximab was 1.06 (95% CI: 1.02–1.10,  $P = 0.005$ ). Patients ( $n = 14$ ) receiving an oral minocycline had a higher serum level of cetuximab than those ( $n = 20$ ) not receiving one ( $P = 0.043$ ), while the co-treatment of minocycline was not associated with the serum levels of sEGFR ( $P = 0.359$ ) and IL-6 ( $P = 0.323$ ) (Fig. S3). All patients used a moisturizing drug or skin care product, and thus the correlations with serum markers were not evaluated. Fourteen patients were prescribed a nail medication for paronychia. The co-treatment of a nail medication

was not associated with the serum levels of cetuximab ( $P = 0.959$ ), sEGFR ( $P = 0.592$ ), and IL-6 ( $P = 0.641$ ).

### **Relationships with hypomagnesemia**

The numbers of patients co-treated with and without intravenous magnesium sulfate were 9 and 25, respectively. The hypomagnesemia treatment was not associated with the serum levels of cetuximab ( $P = 0.514$ ), sEGFR ( $P = 0.591$ ), and IL-6 ( $P = 0.848$ ) (Fig. S4). The serum level of magnesium was not correlated with that of cetuximab ( $R^2 = 0.048$ ,  $P = 0.293$ ), sEGFR ( $R^2 = 0.004$ ,  $P = 0.758$ ), and IL-6 ( $R^2 = 0.046$ ,  $P = 0.301$ ) in 25 patients not receiving the hypomagnesemia treatment.

### **Discussion**

EGFR activation potentially alters blood circulating levels of endogenous markers including soluble receptors and cytokines in cancer patients. To the best of our knowledge, this is the first report that has characterized the correlations between serum EGFR-related markers, skin disorders, and their medications based on serum cetuximab in head and neck cancer patients.

Serum cetuximab did not significantly increase after week 4 in the study patients. In an earlier study, the serum level of cetuximab at week 4 was found to be similar to that at week 8 in head and neck cancer patients [37]. Serum cetuximab had a half-life of 4 days in Japanese cancer patients and reached a steady state after 3 doses of cetuximab administration [38,39].

Cetuximab-induced skin rash began within 25 days in approximately 80% of cancer patients receiving intravenous cetuximab [29]. Thus, the present study evaluated the associations between serum cetuximab and EGFR-related markers and skin disorders after week 4.

The serum sEGFR level only at week 2 was lower than that at week 1. Cetuximab inhibits the release of sEGFR from the surface of cancer cells [20]. Pool et al. reported that anti-EGFR antibody bound to sEGFR and that the complex was eliminated by hepatic metabolism [40]. Liver uptake of the complex was saturated by increasing the anti-EGFR antibody dose, and thus blood level of the antibody was elevated. The decrease of serum sEGFR at week 2 may indicate the low production and elevated elimination of sEGFR. Saturation of hepatic elimination of cetuximab/sEGFR complex potentially reduced the differences in serum sEGFR between baseline and weeks 3–6.

Serum IL-6 levels did not show time-dependent changes in weeks 1–6. Cetuximab is thought to suppress IL-6 secretion through the downstream JAK2/STAT3 signal in cancer cells [25,26]. However, a direct effect of cetuximab administration on serum IL-6 was not observed. The IQR of serum IL-6 (13.3–18.7 pg/mL) at week 2 was narrower than that (12.7–30.6 pg/mL) at week 1. Contrary to expectations, the degree of serum IL-6 variation (IQR, 14.8–51.2 pg/mL) at week 6 was greater than week 1 and 2. In an earlier study, a time-dependent increase in serum IL-6 was observed in chemotherapy-treated head and neck cancer patients who presented early disease progression [41]. The serum IL-6 was potentially varied by cetuximab-induced suppression and disease-related elevation in the present study.

A negative association was observed between serum levels of cetuximab and IL-6 at week 4 or later, even though serum IL-6 showed inter- and intra-individual variation. Cetuximab is catabolized by lysosomal degradation in endothelial cells of various organs [42]. Cancer patients with higher serum pro-inflammatory cytokine levels showed more enhanced catabolism of endogenous protein [43]. The present data suggest that serum cetuximab is readily eliminated in patients with systemic tumor-induced inflammation. In contrast, these results could not distinguish cetuximab-related IL-6 suppression from inflammation-promoting cetuximab elimination. Serum IL-6 is a potential marker associated with serum cetuximab in cancer patients.

Serum sEGFR was not correlated with serum cetuximab. The IQR of serum sEGFR was 20.5–29.4 ng/mL in these head and neck cancer patients. In an earlier study, oral cancer patients without cetuximab therapy also had a small variation in serum sEGFR (IQR, 41.3–51.9 ng/mL) [17]. Serum sEGFR in our study patients receiving intravenous cetuximab was lower than this previous study and its degree of variation at week 4 or later was similar. Serum sEGFR may not be an associated marker for serum cetuximab. In contrast, a positive correlation was observed between serum sEGFR and IL-6. The serum level of CRP, a serum inflammatory marker, was also positively associated with that of sEGFR and IL-6 (Fig. S1). The serum sEGFR and IL-6 were elevated according to the disease progression in cancer patients [9,18]. The relationship between serum sEGFR and IL-6 appeared to be an indirect correlation reflecting elevated systemic inflammation. These results suggest that tumor-induced

inflammation determines the serum levels of sEGFR and IL-6 in advanced cancer patients. Serum sEGFR as well as IL-6 is a possible inflammation-related marker during cetuximab treatment.

The present study evaluated the associations between serum cetuximab and three types of skin disorders. With regard to skin rash, the serum cetuximab level was 2-fold higher in grade 2–3 patients than in grade 0–1 patients. The excretion rate of serum cetuximab was reported to be partially responsible for cetuximab-induced skin toxicity in colorectal cancer patients [31]. In the present study, the cut-off value of serum cetuximab related to severe skin rash was 71 µg/mL with its sensitivity of 59%. Approximately 40% of the patients with severe skin rash had serum cetuximab of < 71 µg/mL, while this symptom was observed in almost all the patients with serum cetuximab of > 71 µg/mL. These results indicate that not only serum cetuximab but also other factors contribute to the development of severe skin rash. In contrast, the elevation of serum cetuximab was identified as a considerable risk factor of severe skin rash based on its OR. The serum level of cetuximab was a factor strongly associated with the incidence of severe skin rash in head and neck cancer patients. Louedec et al. reported that head and neck cancer patients with higher pre-dose serum cetuximab levels had longer progression-free survival [44]. Further studies including clinical efficacy analyses would reveal the serum cetuximab level that achieves an anti-cancer effect. In contrast, serum sEGFR and IL-6 had no associations with the skin disorders severities in the present study. The changes of the serum EGFR-related markers may not reflect the cetuximab-induced skin toxicity.

This study investigated the associations between serum cetuximab and skin treatments provided by one dermatologist who was blinded to serum markers levels. The serum cetuximab level was 2-fold higher in the patients co-treated for skin rash with a stronger topical corticosteroid than with a medium potency topical corticosteroid or nonusers. The stronger topical corticosteroids were applied to the patients having serum cetuximab level of  $> 76$   $\mu\text{g/mL}$ . The logistic regression analysis demonstrated that the elevation of serum cetuximab was a factor enhancing the potency of topical corticosteroid. Additionally, cancer patients concomitantly receiving a minocycline had a higher serum cetuximab level. These data suggest that serum cetuximab may be a clinical marker with which to predict the necessity for appropriate skin treatments for skin rash. In contrast, the severities of dry skin and paronychia and their treatments had no correlation to the serum cetuximab level. The dry skin observed was potentially affected by various factors including aging, the usage of skin care products, and previous therapy with a cytotoxic drug [28]. Paronychia mainly emerges on the toes and is worsened by external irritations to the nails [28]. The activities of the present study patients might have had an influence on the severity of the paronychia that developed.

Serum cetuximab and EGFR-related markers had no associations with the hypomagnesemia treatment and serum magnesium in the study patients. The EGFR activation promotes the expression of transient receptor potential melastatin 6 (TRPM6) channel involved in renal reabsorption of magnesium. Cetuximab induces the hypomagnesemia by reducing TRPM6 expression through EGFR inhibition in the distal renal tubules [34]. Serum cetuximab

exposure and change of EGFR signal were expected to influence renal reabsorption of magnesium. However, the serum cetuximab and EGFR-related markers were not associated with the incidence of hypomagnesemia.

The present study has several limitations. First, we enrolled patients with locally advanced, recurrent, and/or distant metastatic head and neck cancer. The patients had various types of primary lesions and were receiving several anti-cancer drugs or radiation therapy. Serum levels of cetuximab and EGFR-related markers were not associated with the primary lesion type, distant metastasis, or chemotherapy regimen. The primary lesion type, distant metastasis, and chemotherapy regimen most likely had little influence on the skin disorders. Future studies would identify the relationship between serum cetuximab and its tumor response by fixing the disease characteristics and cetuximab therapy. Second, the observation period in the present study was weeks 4–161. Serum sEGFR and IL-6 levels potentially change in association with cancer progression. Skin rash has been reported to emerge at the beginning of cetuximab treatment and continue over the treatment period, with the exception of a small proportion of patients with spontaneous improvement [29]. This study evaluated the relationships between serum cetuximab and markers, and skin disorders after serum cetuximab had reached the steady state. In contrast, dry skin and paronychia emerged at weeks 5–9 of administration and progressed more slowly than skin rash [28]. The dry skin and paronychia need to be evaluated at multiple points after week 5 with an interval of about 1 month. We may be able to determine the effect of serum cetuximab on the severities of these skin disorders

during long observation period. Third, the present study could not identify the serum concentration profile of cetuximab with several blood sampling points. A population pharmacokinetic model could be utilized to understand the magnitude of serum IL-6 effect on the increased cetuximab clearance. Additionally, the analyses are useful for providing more detailed relationships between serum cetuximab and EGFR-related markers, and skin disorders. Further study including the serum cetuximab exposure-response analyses would confirm our findings and their implications. Fourth, this study had the low statistical power of 0.52 in the correlation analyses between serum cetuximab and EGFR-related markers. In contrast, the analyses for associations of serum cetuximab with skin rash severity and its medication possessed the sufficient statistical power of 0.81 and 0.83, respectively. Additionally, the present study performed at a single institution. Further study with a large number of patients at multiple institutions needs to highlight the correlations between serum markers and reduce the probability of type II error in the analysis.

The study patients exhibited a large variation in serum cetuximab (IQR, 34.3–83.2  $\mu\text{g/mL}$ ). To achieve an anti-cancer effect, the serum cetuximab level may be excessive or insufficient in some of the patients. The skin rash of grade 2–3 is expected to become evident at pre-dose serum cetuximab level of  $> 71 \mu\text{g/mL}$  in cancer patients. Serum cetuximab cut-off value of  $76 \mu\text{g/mL}$  may be useful to prevent the severe skin rash using stronger topical corticosteroids. Depending on the elevation of  $10 \mu\text{g/mL}$  serum cetuximab, the risk of the severe skin rash and its skin treatment using stronger topical corticosteroid was increased by 1.41- and

1.76-fold, respectively. In contrast, serum cetuximab levels have not been extensively evaluated at multiple institutions and need to be predictable. Serum IL-6 can be routinely measured by a clinical laboratory test and be used as a conventional marker of inflammation. Our study suggests that serum IL-6 is a predictable factor for serum cetuximab exposure. The clinical management of the serum cetuximab level would contribute to the optimization of the balance of its anti-cancer effect and skin disorder tolerance in advanced cancer patients.

## **Conclusions**

The present head and neck cancer patients with a higher serum IL-6 level tended to have a lower serum cetuximab level. The serum level of cetuximab had positive correlations to skin rash severity and its medication in the study population.

## **Declarations**

## **Funding**

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## **Availability of data and materials**

The data that support the findings of the present study are available from the corresponding author upon reasonable request.

### **Code availability**

Not applicable

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### **Authors' contributions**

Ka.S. and T.N. conceptualized the study with input from Sa.H. and J.K. Ka.S. and T.N. funded the study. Ka.S. and Ko.S. recruited patients and performed blood sampling with assistance from Se.H. and H.M. Ka.S. measured drug and biomarker levels. Sa.H. evaluated clinical symptoms. Ka.S. curated drug and biomarker level results and analyzed and interpreted data with assistance of T.N. and Sa.H. Ka.S. and T.N. wrote the manuscript and all coauthors reviewed and contributed to the manuscript.

### **Compliance with ethical standards**

**Conflicts of interest**

The authors declare there are no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

**Ethics approval**

The present study was conducted in accordance with the Declaration of Helsinki, its amendments, and the Ethical Guidelines for Medical and Health Research Involving Human Subjects in Japan. The study protocol was approved by the Ethics Committee of Hamamatsu University School of Medicine. All patients were fully informed of the scientific aim of this study and each patient provided written informed consent before study enrollment.

**Consent for publication**

Not applicable

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## Figure legends

**Fig. 1** Serum levels of cetuximab and epidermal growth factor receptor (EGFR)-related markers in 11 head and neck cancer patients

(a) cetuximab, (b) soluble EGFR (sEGFR), and (c) interleukin-6 (IL-6). Box plots represent the median, 25th, and 75th percentiles. The whiskers indicate the range and extend to within 1.5 times the length of the inner quartiles. Differences in serum levels of cetuximab and EGFR-related markers between administration weeks were evaluated using the Kruskal-Wallis test and Mann-Whitney *U* test with the Bonferroni correction. \*,  $P < 0.05$ .

**Fig. 2** Relationships between serum levels of cetuximab and epidermal growth factor receptor (EGFR)-related markers at week 4 or later in head and neck cancer patients

Serum levels of (a) cetuximab versus interleukin-6 (IL-6), (b) cetuximab versus soluble EGFR (sEGFR), and (c) sEGFR versus IL-6. The correlations were evaluated using the Pearson correlation coefficient.

**Fig. 3** Relationships between skin rash severity and serum levels of cetuximab or epidermal growth factor receptor (EGFR)-related markers in head and neck cancer patients

The associations of skin rash severity (grade 0–1,  $n = 17$ ; grade 2–3,  $n = 17$ ) with cetuximab (a), soluble EGFR (sEGFR) (b), and interleukin-6 (IL-6) (c). (d) Receiver operating characteristic (ROC) curve of serum cetuximab related to a grade 2–3 skin rash. The severities

of skin disorders were assessed using the CTCAE ver. 4.0 grading system. Box plots represent the median, 25th, and 75th percentiles. The whiskers indicate the range and extend to within 1.5 times the length of the inner quartiles. The relationships were analyzed using the Mann-Whitney *U* test. \*,  $P < 0.05$ . The solid line and dotted line are the ROC curve of serum cetuximab and reference line, respectively. AUC, area under the ROC curve.

**Fig. 4** Relationships between topical corticosteroid potency and serum levels of cetuximab or epidermal growth factor receptor (EGFR)-related markers in head and neck cancer patients

The associations of topical corticosteroid potency with cetuximab (a), soluble EGFR (sEGFR) (b), and interleukin-6 (IL-6) (c). (d) Receiver operating characteristic (ROC) curve of serum cetuximab related to skin rash treatment using strong, very strong, or strongest topical corticosteroid. The potency of topical corticosteroids used for skin rash was medium (M,  $n = 13$ ), strong (S,  $n = 1$ ), very strong (VS,  $n = 13$ ), or strongest (SG,  $n = 1$ ). Six patients were nonusers of topical corticosteroids (N). Box plots represent the median, 25th, and 75th percentiles. The whiskers indicate the range and extend to within 1.5 times the length of the inner quartiles. The relationships were analyzed using the Mann-Whitney *U* test. \*\*,  $P < 0.01$ . The solid line and dotted line are the ROC curve of serum cetuximab and reference line, respectively. AUC, area under the ROC curve.

## Supplementary Figure legends

**Fig. S1** Correlations between serum levels of CRP and cetuximab (a), sEGFR (b), or IL-6 (c) in head and neck cancer patients

The correlations were evaluated using the Pearson correlation coefficient.

**Fig. S2** Relationships between skin disorders and serum cetuximab or EGFR-related biomarkers in head and neck cancer patients

(a) Relationships between dry skin severity and serum levels of cetuximab (a-1), sEGFR (a-2), or IL-6 (a-3). (b) Relationships between paronychia severity and serum levels of cetuximab (b-1), sEGFR (b-2), or IL-6 (b-3). The numbers of patients with dry skin of grade 0, 1, 2, and 3 were 0, 25, 9, and 0, respectively. The numbers of patients with paronychia of grade 0, 1, 2, and 3 were 15, 9, 10, and 0, respectively. Box plots represent the median, 25th, and 75th percentiles. The whiskers indicate the range and extend to within 1.5 times the length of the inner quartiles. The relationships were analyzed using the Mann-Whitney *U* test.

**Fig. S3** Relationships between co-treatments of skin medications and serum cetuximab or EGFR-related biomarkers in head and neck cancer patients

(a) Relationships between minocycline treatment and serum levels of cetuximab (a-1), sEGFR (a-2), or IL-6 (a-3). (b) Relationships between nail medication and serum levels of cetuximab (b-1), sEGFR (b-2), or IL-6 (b-3). Box plots represent the median, 25th, and 75th percentiles.

The whiskers indicate the range and extend to within 1.5 times the length of the inner quartiles.

The relationships were analyzed using the Mann-Whitney *U* test.

**Fig. S4** Relationships between serum cetuximab or EGFR-related biomarkers and hypomagnesemia in head and neck cancer patients

(a) Relationships between serum levels of cetuximab (a-1), sEGFR (a-2), or IL-6 (a-3) and hypomagnesemia treatment. (b) Correlations between serum levels of cetuximab (b-1), sEGFR (b-2), or IL-6 (b-3) and magnesium in 25 patients without hypomagnesemia treatment. Box plots represent the median, 25th, and 75th percentiles. The whiskers indicate the range and extend to within 1.5 times the length of the inner quartiles. The relationships with hypomagnesemia treatment were analyzed using the Mann-Whitney *U* test. The correlations with serum magnesium were evaluated using the Pearson correlation coefficient.

**Table 1** Patient characteristics in the study population

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Gender, male/female	34, 26/8
Age, years	66 (63–70)
Body weight, kg	49.6 (42.0–56.8)
Body surface area, m <sup>2</sup>	1.52 (1.41–1.68)
Serum total protein, g/dL	6.3 (6.0–6.6)
Serum albumin, g/dL	3.6 (3.1–3.8)
Serum creatinine, mg/dL	0.73 (0.59–1.00)
Blood urea nitrogen, mg/dL	15.5 (12.4–20.3)
Serum total bilirubin, mg/dL	0.5 (0.4–0.6)
Aspartate aminotransferase, IU/L	21 (17–27)
Alanine aminotransferase, IU/L	18 (14–27)
Serum C-reactive protein, mg/dL	0.81 (0.27–2.34)
Cancer type	
Hypopharyngeal	9
Oral	9
Oropharyngeal	5
Maxillary sinus	5
Laryngeal	2
Other	4
Cancer stage, I/II/III/IV	0/0/1/33
Distant metastasis, with/without	18/16
Cetuximab treatment period, weeks	7 (4–21)
Chemotherapy regimen	
Cetuximab monotherapy	10
Cetuximab + cisplatin + 5-fluorouracil	7
Cetuximab + paclitaxel	11
Cetuximab + radiation	6

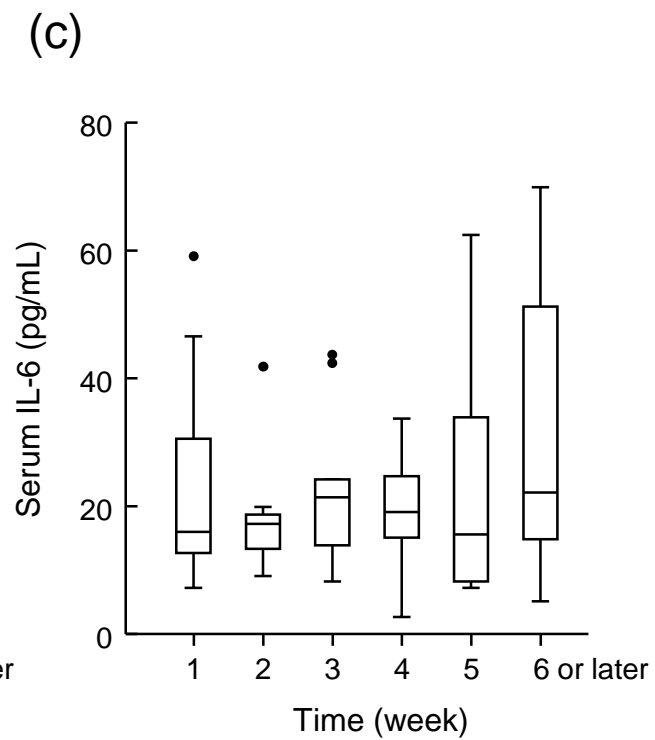
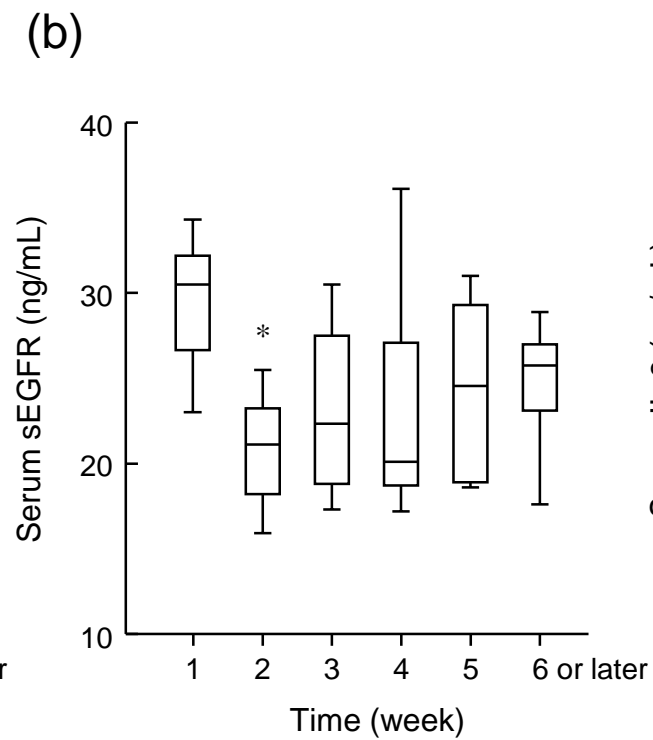
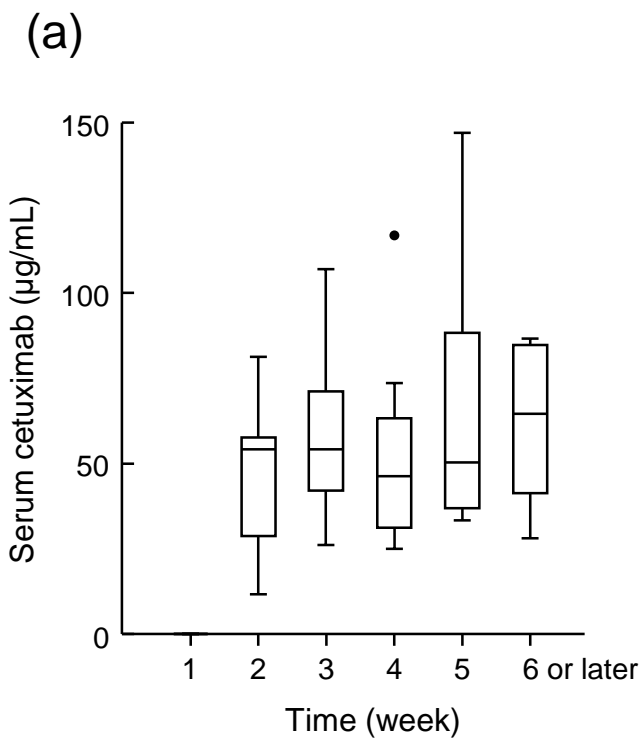
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Data are expressed as the median with interquartile range in parentheses.

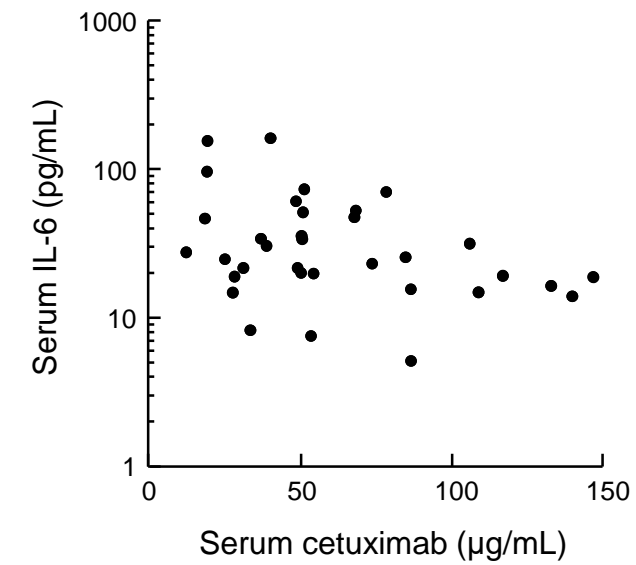
**Table S1** Clinical classification and grading system of cetuximab-induced skin disorders in the present study

Skin disorder		Severity		
		Grade 1	Grade 2	Grade 3
Skin rash	Generalized symptom	Papules and/or pustules covering <10% BSA without symptoms of pruritus or tenderness.	Papules and/or pustules covering <30% BSA, with any symptom of pruritus or tenderness.	Papules and/or pustules covering >30% BSA, which may or may not be associated with symptoms of pruritus or tenderness.
	Scalp	Mild eczematous change, and papules and/or pustules without pruritus. Asymptomatic or mild symptoms which do not affect social circumstances by the patient.	Moderate eczematous lesions, and papules and/or pustules with symptoms of pruritus. A psychosocial worry may occur, which cause communication problem.	Severe eczematous lesions, and dense papules and/or pustules. Skin ulceration may occur with substantial exudate.
	Face			Irritation may occur due to bleeding and/or scratch. A psychosocial worry may occur, which cause remarkable communication problem.
	Trunk and extremities	Papules and/or pustules without itch.	Papules and/or pustules with moderate pruritus occasionally accompanied by inflammatory skin changes.	Dense papules and/or pustules with severe pruritus which may interfere constant sleep.
Dry skin		Mild dry skin without pruritus.	Moderate dry skin with itch. The affected skin may be reflected by scales from tiny to small-sized scales.	Severe dry skin covered by flaky and dull, medium to large-sized scales. The affected skin may develop pruritic erythema or asteatotic eczema.
Paronychia		Painless nail fold erythema with disruption of the cuticle.	Painful nail fold edema or erythema associated with fissure, discharge, bleeding, or nail plate separation. These disorders interfere with instrumental ADL.	Severely painful nail fold changes accompanied by granulation tissue formation. These disorders interfere with self-care ADL.

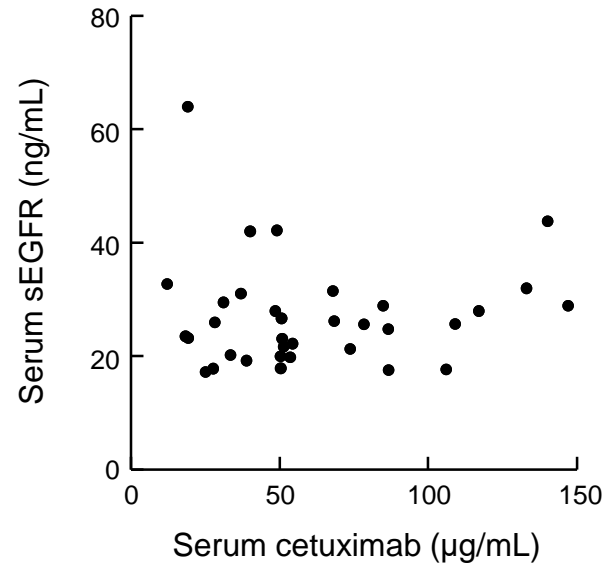
The grading system for skin rash, dry skin, and paronychia created by the National Hospital Organization Shikoku Cancer Center. These criteria are a modified version of the Common Terminology Criteria for Adverse Events (CTCAE ver. 4.0) and focus on subjective symptoms in cancer patients. Each skin disorder without any symptoms was evaluated as grade zero. Generalized and localized rashes were respectively evaluated as severity grade from one to three, and then the most severe grade among those categories represented the physiologic status. BSA, body surface area and ADL, activities of daily living.



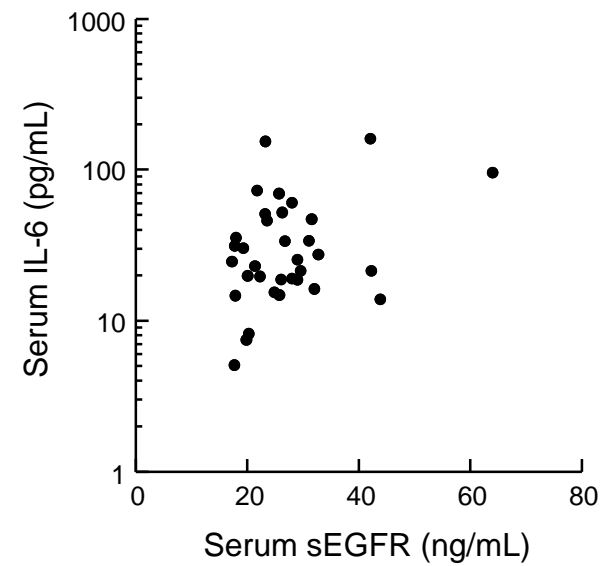
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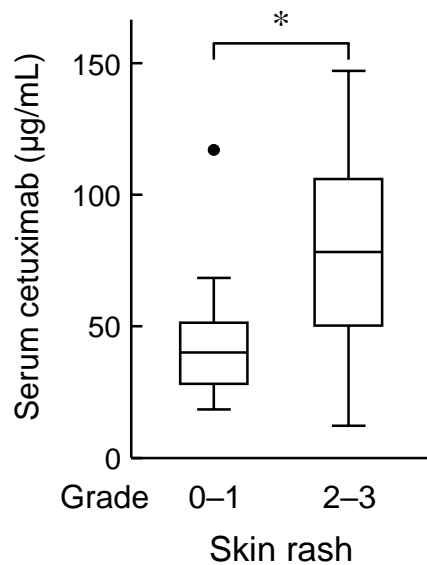
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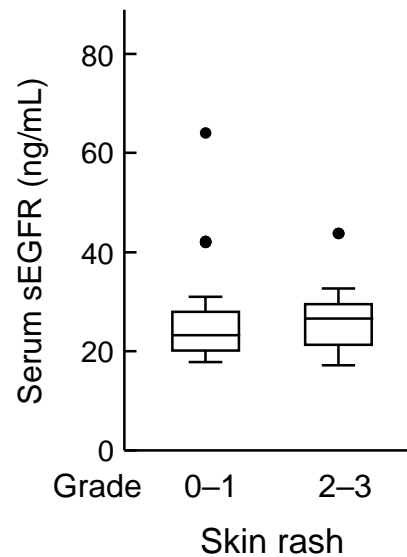
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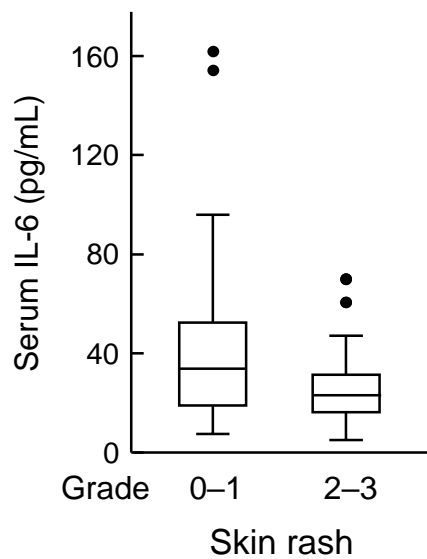
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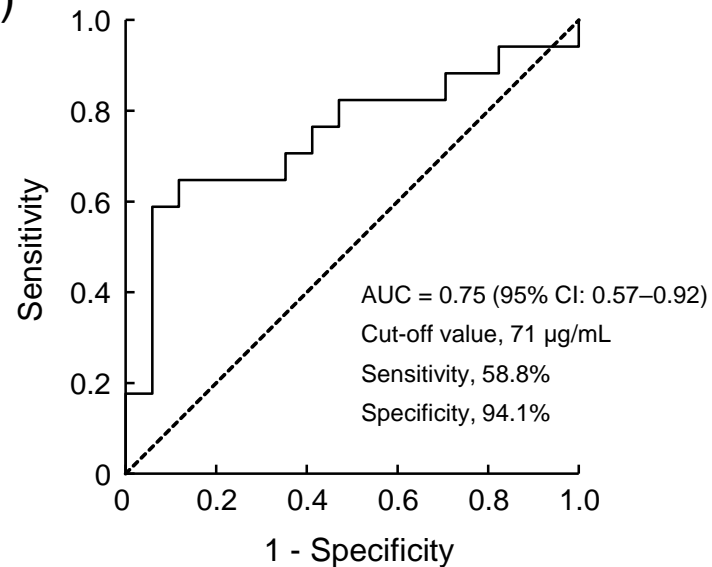
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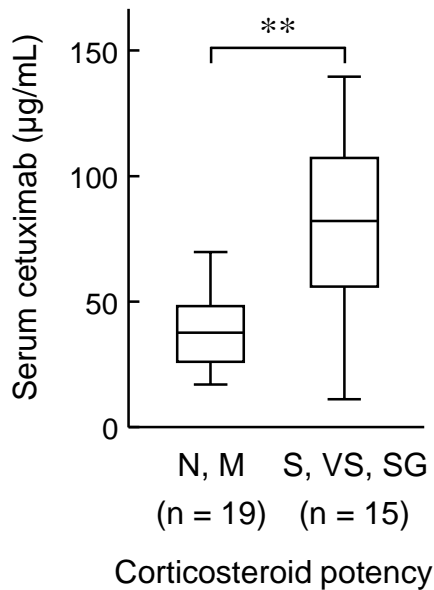
(c)



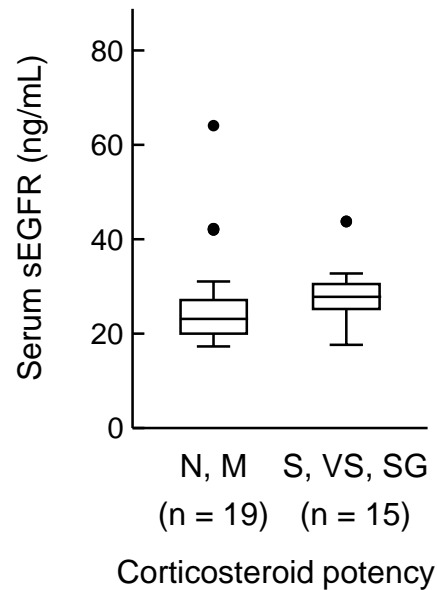
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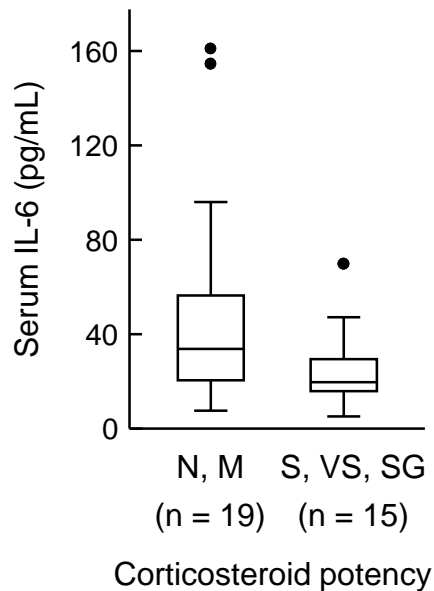
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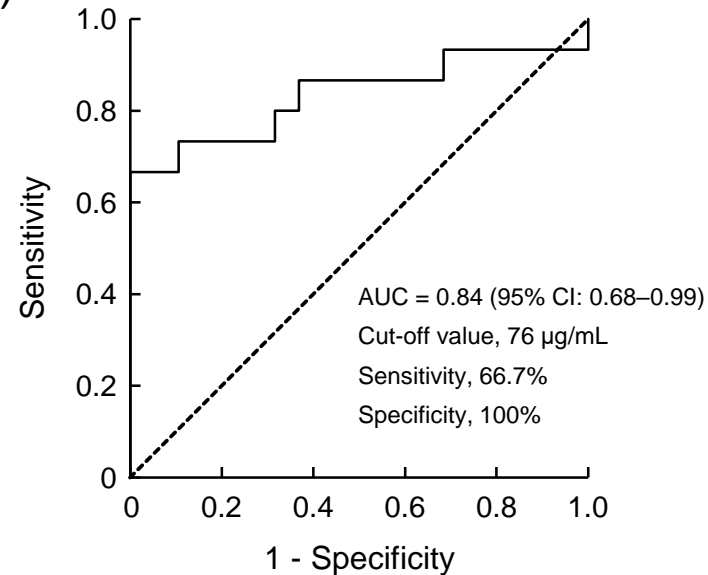
(b)



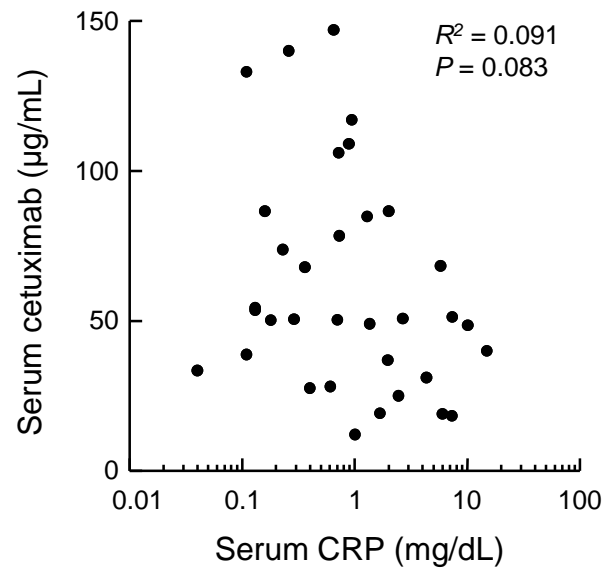
(c)



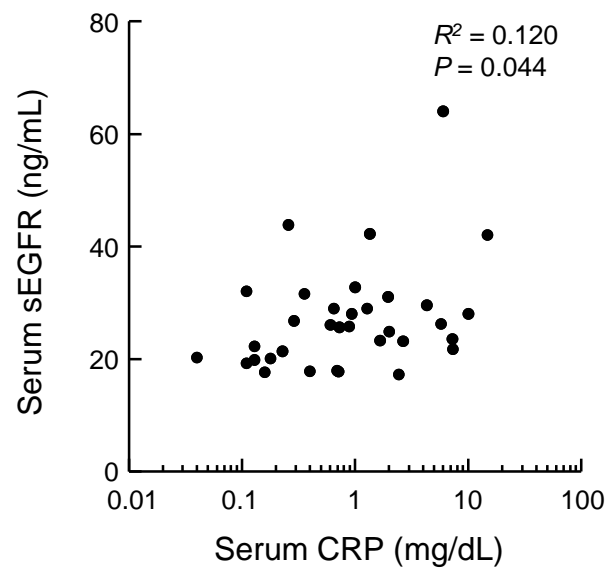
(d)



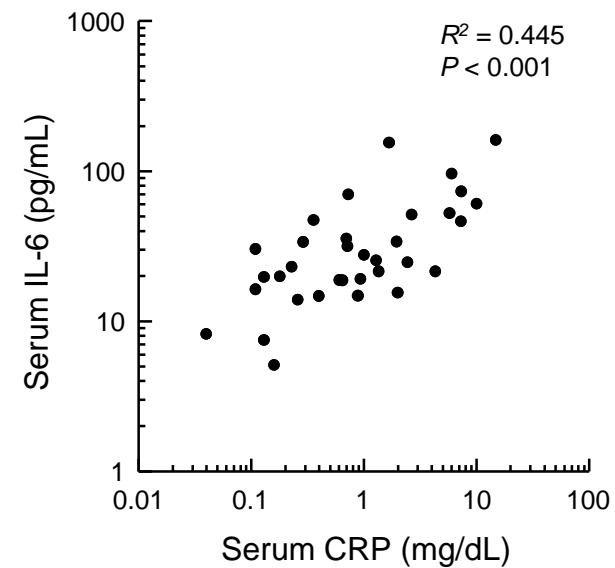
(a)



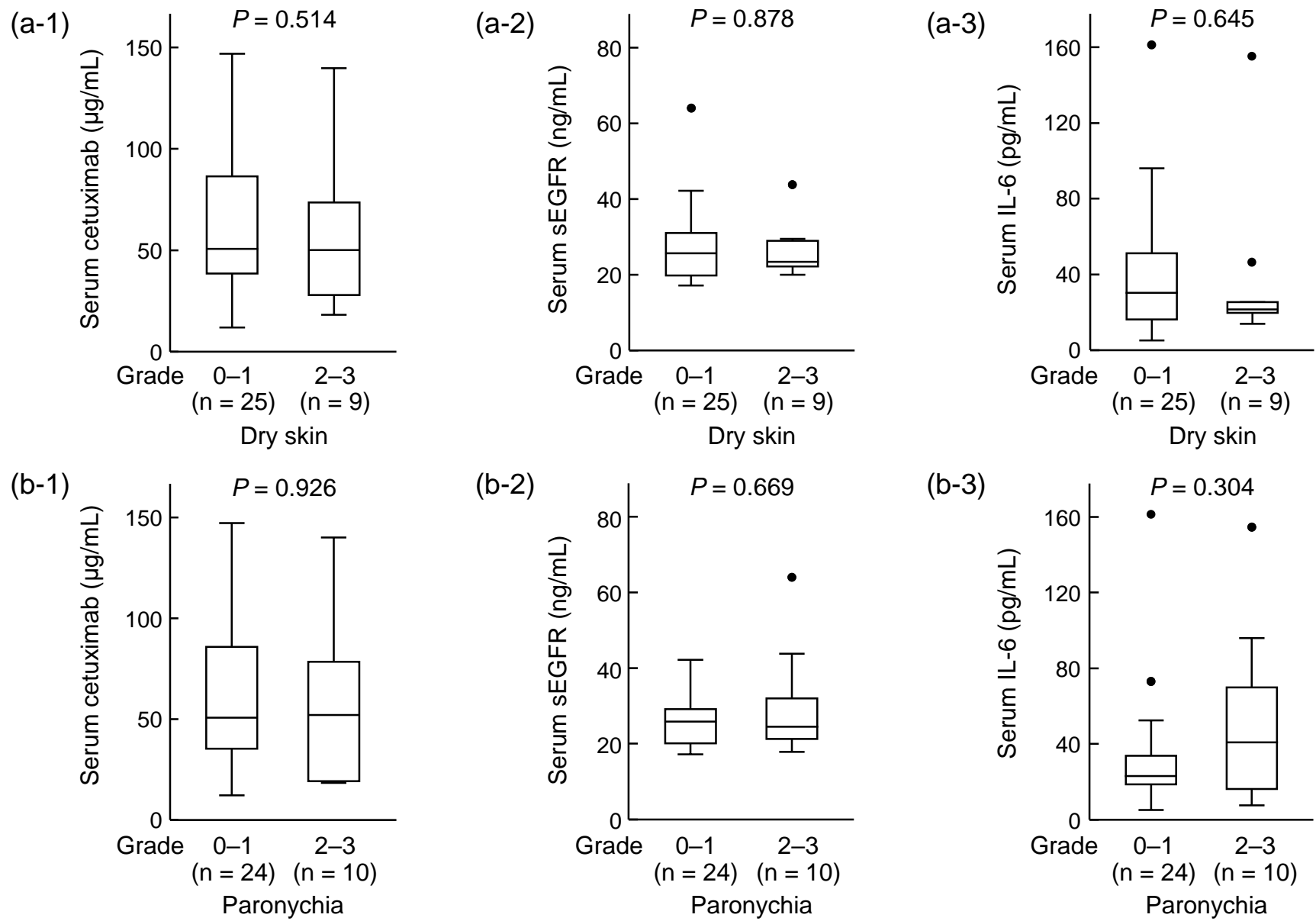
(b)



(c)

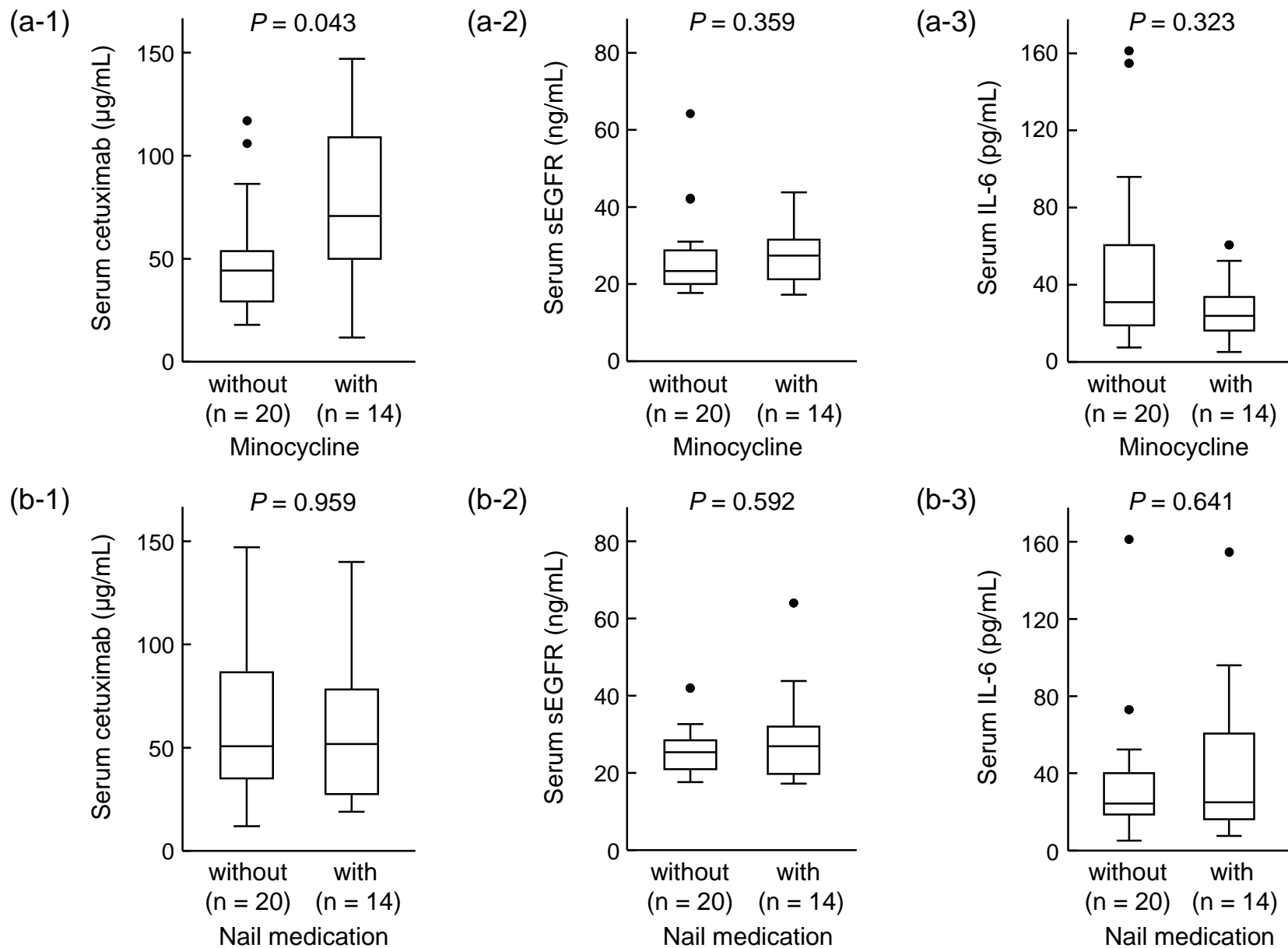


**Fig. S1** Correlations between serum levels of CRP and cetuximab (a), sEGFR (b), or IL-6 (c) in head and neck cancer patients. The correlations were evaluated using the Pearson correlation coefficient.

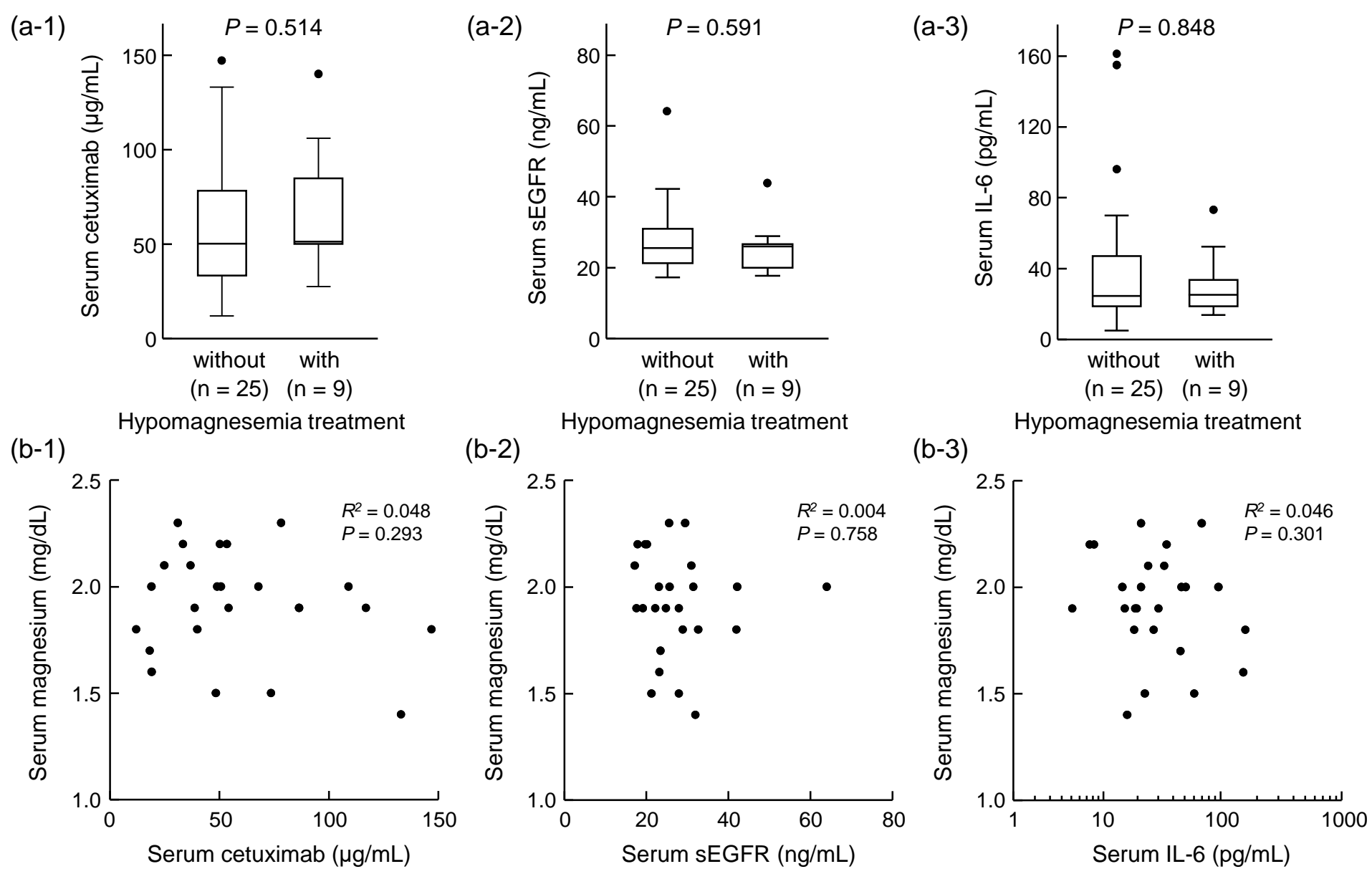


**Fig. S2** Relationships between skin disorders and serum cetuximab or EGFR-related biomarkers in head and neck cancer patients

(a) Relationships between dry skin severity and serum levels of cetuximab (a-1), sEGFR (a-2), or IL-6 (a-3). (b) Relationships between paronychia severity and serum levels of cetuximab (b-1), sEGFR (b-2), or IL-6 (b-3). The numbers of patients with dry skin of grade 0, 1, 2, and 3 were 0, 25, 9, and 0, respectively. The numbers of patients with paronychia of grade 0, 1, 2, and 3 were 15, 9, 10, and 0, respectively. Box plots represent the median, 25th, and 75th percentiles. The whiskers indicate the range and extend to within 1.5 times the length of the inner quartiles. The relationships were analyzed using the Mann-Whitney  $U$  test.



**Fig. S3** Relationships between co-treatments of skin medications and serum cetuximab or EGFR-related biomarkers in head and neck cancer patients (a) Relationships between minocycline treatment and serum levels of cetuximab (a-1), sEGFR (a-2), or IL-6 (a-3). (b) Relationships between nail medication and serum levels of cetuximab (b-1), sEGFR (b-2), or IL-6 (b-3). Box plots represent the median, 25th, and 75th percentiles. The whiskers indicate the range and extend to within 1.5 times the length of the inner quartiles. The relationships were analyzed using the Mann-Whitney  $U$  test.



**Fig. S4** Relationships between serum cetuximab or EGFR-related biomarkers and hypomagnesemia in head and neck cancer patients

(a) Relationships between serum levels of cetuximab (a-1), sEGFR (a-2), or IL-6 (a-3) and hypomagnesemia treatment. (b) Correlations between serum levels of cetuximab (b-1), sEGFR (b-2), or IL-6 (b-3) and magnesium in 25 patients without hypomagnesemia treatment. Box plots represent the median, 25th, and 75th percentiles. The whiskers indicate the range and extend to within 1.5 times the length of the inner quartiles. The relationships with hypomagnesemia treatment were analyzed using the Mann-Whitney  $U$  test. The correlations with serum magnesium were evaluated using the Pearson correlation coefficient.