Supplementary material has been published as submitted. It has not been copyedited, or typeset by Acta Dermato-Venereologica

Supplementary methods

S1. Detailed study design and setting

This clinical study comprised a cross-sectional study (STEP 1 and STEP 2, shown in Fig. 1) and a prospective cohort study (STEP 3, shown in Fig. 1). In STEP 1, a cross-sectional comparison of spontaneous itch, MA, and skin dryness was made between healthy controls (n = 30) and patients with AD (n = 32). In STEP 2, a cross-sectional sub-analysis was conducted to examine correlations between MA scores and disease-related parameters (EASI, Eos, IgE, and TARC) in AD patients (n = 24). In STEP 3, a prospective cohort analysis was conducted to evaluate the effects of dupilumab treatment on MA and serum biomarkers (Eos, IgE, and TARC) in AD patients who selected dupilumab treatment (n = 17 at enrollment), of whom 10 completed all scheduled visits and were included in the final analysis. Participant recruitment was conducted continuously from April 2021 through March 2025. For STEP 1 and STEP 2, all assessments were performed at a single visit. For STEP 3, follow-up continued from the 1st to the 9th administration of dupilumab (approximately 5 months after treatment initiation). This study was conducted in the outpatient dermatology clinic at Juntendo University Urayasu Hospital and the Juntendo Itch Research Center conference room located in the same hospital (Urayasu, Chiba, Japan). Measurements were performed under air-conditioned conditions (room temperature 20–25°C, humidity 40–60%) without special climate control.

S2. Participants and eligibility criteria

Human participants included outpatients and hospitalized patients at the Department of Dermatology, hospital staff, and related parties at Juntendo University Urayasu Hospital, who provided written informed consent. Eligibility criteria for each STEP were as follows:

STEP 1 (cross-sectional study): Inclusion criteria were: 1) age 20–89 years; 2) deemed suitable for participation by the attending dermatologist; and 3) provision of written informed consent. Exclusion criteria were: 1) those whom attending physicians deemed unsuitable for study participation; and 2) those unable to provide consent to participate. Patients were diagnosed with atopic dermatitis (AD) according to the criteria of Hanifin and Rajka (S1). Healthy controls had no history of chronic skin disease or systemic inflammatory disorders.

STEP 2 (cross-sectional sub-analysis): STEP 2 included AD patients from STEP 1 who met the same eligibility criteria and underwent routine blood sampling for Eos, IgE, and TARC as part of

standard clinical care. No additional inclusion or exclusion criteria were applied beyond those for STEP 1.

STEP 3 (prospective cohort study): STEP 3 enrolled AD patients from STEP 2 who elected to receive dupilumab treatment because standard topical therapy was insufficient. Inclusion required the ability to attend regular follow-up visits and complete at least nine administrations of dupilumab.

S3. Variables and outcomes

All primary and secondary outcomes, exposures, and related variables were defined in accordance with the recommendations of the STROBE statement. The primary outcome was the MA score, and secondary outcomes included spontaneous itch intensity, skin dryness, and disease-related parameters. All variables, including outcomes, exposures, predictors, potential confounders, and effect modifiers, are summarized in Table S1.

S4. Assessment of spontaneous itch, MA, and skin dryness

Spontaneous itch intensity was assessed by asking AD patients to rate the level of itch at the lesional skin site using a numerical rating scale (NRS) ranging from 0 (no pruritus) to 10 (worst imaginable pruritus) before mechanical stimulation. For healthy control areas and non-lesional skin areas of AD patients, sites with a spontaneous itch level of 0 were selected. MA levels were also quantified using the NRS after applying von Frey filaments (Bioseb, Chaville, France) to the lesional or non-lesional site of AD patients or the volar forearm of healthy controls. At each stimulation, participants were instructed to rate the itch elicited by that mechanical stimulus itself, rather than their pre-existing spontaneous itch, as far as possible. Based on previous reports, von Frey filaments with bending forces of 5.8, 9.8, 13.7, and 19.6 mN were used. One lesional and one non-lesional site were selected for each AD patient, and one forearm site for each healthy control. All filaments were applied in ascending order of bending force. Each filament was applied 3 times to each site, with at least 10 s between applications. Unless otherwise noted, MA data for AD patients are shown for the lesional site. The non-lesional area was positioned as symmetrically as possible to the lesional area. Measurements of the lesional skin area were set at the borders of the lesion. Skin dryness was evaluated by measuring TEWL and SC hydration at the MA measurement sites using VapoMeter® (Delfin Technologies, Kuopio, Finland) and MoistureMeter SC Compact® (Delfin Technologies), respectively.

1

S5. Evaluation of disease-related parameters and dupilumab treatment

EASI scores, which represent the severity of AD, were evaluated according to a previously reported method (S2). Eosinophils were counted using an automatic analyzer. Serum levels of total IgE were measured using electrochemiluminescence, and TARC levels were assessed using a chemiluminescent enzyme immunoassay. The levels of Eos, IgE, and TARC were measured from 6 ml of blood drawn from 24 AD patients. Among them, 17 patients elected to receive dupilumab treatment, and 10 of these completed at least nine administrations and were included in the final analysis of treatment effects. For these 10 patients, changes in Eos, serum IgE, and TARC levels were evaluated before and after dupilumab administration, at the initial and 9th visit, respectively.

According to the Japanese guidelines for the use of dupilumab, 17 patients with AD who showed insufficient response to standard treatments initiated dupilumab therapy. Among them, 10 patients who completed at least nine administrations at our facility were included in the final analysis. They received a loading dose of 600 mg subcutaneously, followed by 300 mg every other week for at least nine administrations.

S6. Bias control and study size

To minimize selection bias, all eligible participants who met the inclusion and exclusion criteria during the recruitment period were consecutively enrolled. Measurement bias was reduced by performing all assessments using standardized devices (VapoMeter®, MoistureMeter SC Compact®, and von Frey filaments) under controlled room conditions (20–25°C, 40–60% humidity). The same trained investigator conducted all measurements throughout the study.

The study size was determined empirically based on the number of eligible participants who met the inclusion criteria and were available during the study period. No formal sample size calculation was performed, as this was an exploratory observational study.

S7. Detailed statistical analysis

Data for participant characteristics (e.g., age, EASI, serum biomarkers) are expressed as the mean ± standard deviation (SD). Data for group comparisons and correlation analyses are presented as the mean and 95% confidence interval (95% CI). Group comparisons were analyzed by two-tailed Student's t-test for two-group comparisons, and by one-way ANOVA for multiple-group comparisons followed by Tukey's or Dunnett's post hoc test. Correlations between NRS scores and skin dryness or disease-related parameters were assessed using Pearson's correlation coefficient, and the statistical significance of each correlation was evaluated by a t-test with n – 2 degrees of freedom. Categorical

variables (e.g., sex distribution) were compared using Fisher's exact test. Quantitative variables (e.g., spontaneous itch level, MA, TEWL, SC hydration, and serum biomarkers) were treated as continuous variables. Statistical analyses were conducted using GraphPad Prism, version 10.0 (GraphPad Software Inc., La Jolla, CA, USA). A p-value < 0.05 was considered statistically significant.

S8. Ethical approval

This study was approved by the Juntendo University ethics committee (approval no. U20-0057) and conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants prior to inclusion in the study.

Supplementary references

S1. Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. Acta Derm Venereol Suppl (Stockh) 1980; 92: 44–47.

S2. Leshem YA, Hajar T, Hanifin JM, Simpson EL. What the eczema area and severity index score tells us about the severity of atopic dermatitis: an interpretability study. Br J Dermatol 2015; 172: 1353-1357.