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Research Paper

Long cardiac troponin T forms in a healthy reference population

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ABSTRACT

Background: Cardiac troponin T (cTnT) is an established biomarker in the diagnosis of myocardial infarction (MI). Recent studies show better discrimination between MI and other conditions when measuring only intact or minimally fragmented cTnT forms (long cTnT), compared to current cTnT assays that measure both intact and highly fragmented cTnT forms (total cTnT). This study investigated the long cTnT concentrations in a healthy population.

Methods: Lithium-heparin plasma samples were collected from 314 healthy volunteers aged 21–85 years (59 % female). The samples were analyzed for total cTnT with Roche Elecsys high sensitivity cTnT assay and with a novel upconversion luminescence-based long cTnT assay.

Results: The median (25th-75th percentile) long cTnT concentration of the reference population was 2.0 (1.3–3.0) ng/l. The 99th percentile URL for the long cTnT assay was 7.3 (95 % confidence interval, 5.6–8.4) ng/l. Of the healthy population 98 % had long cTnT levels above the detection limit of the assay (0.4 ng/l). The imprecision (coefficient of variation) of the long cTnT assay at the 99th percentile URL was 5.1 %. While total cTnT was heavily associated with age, especially in the older population, long cTnT remained low regardless of age. Common comorbidities and medications were also associated with the total cTnT concentration but not with the long cTnT concentration.

Conclusions: The long cTnT assay fulfills the requirements for a high sensitivity cTnT assay. Healthy individuals have low long cTnT concentrations regardless of age and common comorbidities.

1. Introduction

Cardiac troponin T (cTnT) is an established biomarker for the diagnosis of myocardial infarction (MI). MI can be suspected if the circulating cTnT concentration exceeds the 99th percentile upper reference limit (URL) of a healthy population [1]. However, the cTnT concentration is often elevated also in other conditions than MI, such as chronic kidney disease, atrial fibrillation and after strenuous exercise [1–4]. In an effort to better discriminate between MI and other conditions, there has recently been interest in the development of immunoassays for specific troponin forms [5–9].

Current high sensitivity cTnT (hs-cTnT) immunoassays target the stable central region of the cTnT molecule and thus detect both intact and highly truncated forms of cTnT (total cTnT) [10] (Fig. 1). While small cTnT fragments seem to be responsible for the troponin elevations in myocardial injury e.g. in renal impairment and after strenuous exercise, recent studies show that circulating cTnT forms in MI patients are mostly intact or mildly fragmented (long cTnT) [5,8,11–13]. We have developed a highly sensitive upconversion luminescence-based assay to exclusively detect these long cTnT forms [9] (Fig. 1). This assay has been shown to be better than a current commercial hs-cTnT assay at discriminating between MI patients and patients with takotsubo syndrome, end-stage renal disease or after running a full or half marathon [9,14,15].

As the troponin decision limits are very low in MI diagnostics, high sensitivity assays should be preferred [1,16–18]. Assays claiming high sensitivity should be able to detect the cTnT concentration of at least 50 % of a healthy population and have a 10 % or less coefficient of variation

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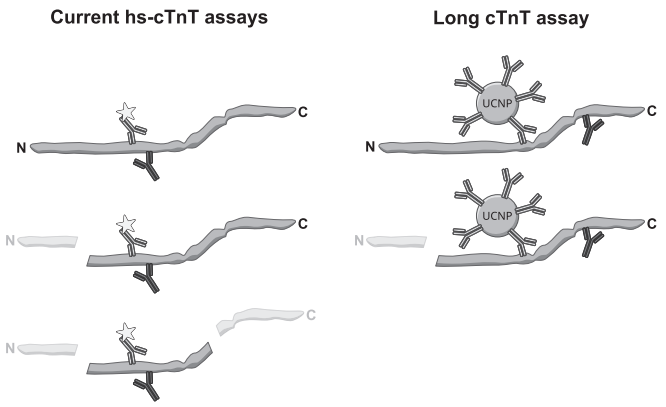

Fig. 1. Cardiac troponin T (cTnT) forms detected by current high sensitivity cTnT assays and the novel upconversion luminescence-based long cTnT assay. The detection antibody is star-labelled in the current hs-cTnT assay, and bound to the surface of an upconverting nanoparticle (UCNP) in the long cTnT assay. Image not to scale.

(CV) imprecision at the 99th percentile URL [1,16–18].

The aim of this study was to determine the long cTnT concentrations in a healthy population and to establish the 99th percentile URL for the upconversion luminescence-based long cTnT assay.

2. Materials and methods

2.1. Study cohort

A total of 314 apparently healthy individuals were recruited as the reference population for the troponin fragmentation in myocardial injury (Tropo-Fragm) study (ClinicalTrials.gov Identifier: NCT04465591). All participants filled out a health and background questionnaire. None of the participants declared a history of coronary artery disease, MI, intermittent claudication, stroke, pacemaker, atrial fibrillation or a kidney disease. All participants were at least 18 years old and gave written informed consent. The study was approved by the Medical Ethics Committee of the Wellbeing Services Country of Southwest Finland (114/1801/2019). The study complies with the Declaration of Helsinki as revised in 2024.

2.2. Clinical samples and sample analysis

Lithium-heparin plasma samples (Vacuette® 9 ml, Greiner Bio-One GmbH, Austria) were collected from participants and prepared according to manufacturer's instructions. The total cTnT was measured fresh after sampling with the Elecsys Troponin T hs kit using the Cobas 8000 system (e801 module) (Roche Diagnostics GmbH, Germany) at the Turku University Hospital laboratory. For long cTnT analyses the samples were stored at −80 °C until batch analysis. The long cTnT concentration was measured from all samples using a previously published protocol [9] with a few modifications. First, the calibrators and samples were analyzed as two replicates instead of three. Second, the capture antibody and sample incubations were 60 min instead of 30 min. These modifications were made to streamline the analysis process and they were experimentally proven not to impact the performance of the assay. The principle of the long cTnT assay and how it differs from the Roche hs-cTnT assay (current hs-cTnT assays) is illustrated in Fig. 1. Briefly, the long cTnT assay detects intact and mildly fragmented forms of cTnT that have not been cleaved at the C-terminal region of amino acid residues 189–223, which contains several cleavage sites. The capture and tracer antibodies in the long cTnT assay bind to epitopes on both sides of the C-terminal cleavage region. The total cTnT assay antibodies recognize epitopes in the stable central part of the cTnT molecule, and thus the assay detects intact cTnT forms as well as mildly and heavily truncated cTnT forms. The limits of detection (LoD) for the total and long cTnT assays are 3.0 ng/l and 0.4 ng/l, and the limits of quantitation (LoQ, 10 % CV) 13 ng/l and 1.8 ng/l, respectively. Concentrations below the LoD were reported as the LoD concentration in all analyses. Troponin ratio was calculated by dividing the result of the long cTnT assay by the result of the Roche hs-cTnT assay [8]. The long cTnT assay is calibrated with native troponin ITC complex (Hytest Ltd, Finland) in buffer while the total cTnT assay uses recombinant cTnT calibrators [9]. A 100 ng/l troponin complex calibrator of the long cTnT assay gives a 55 ng/l result with the total cTnT assay. However, the assays may detect cTnT forms differently in buffer and in heparin plasma.

2.3. Statistical analysis

The 99th percentile URL was determined with the non-parametric approach, after excluding outliers with the Dixon-Reed method, for the whole group and separately for males and females, as recommended in the Clinical and Laboratory Standards Institute (CLSI) document EP28-A3c [19]. The 90 % and 95 % confidence intervals (CI) for the 99th percentile URLs were determined by bootstrapping, as suggested in the International Federation of Clinical Chemistry (IFCC) special report [16], using 10,000 repetitions.

Pearson correlation was used to assess correlation between variables. Linear regression was used to study the relationship between age and cTnT concentrations. Mann-Whitney *U* test was used to compare groups. The normality assumption was assessed visually using histograms and Q-Q plots, as well as numerically with skewness and kurtosis values and the Shapiro-Wilk test. All analyses were performed as two-tailed and a significance level < 0.05 was considered significant. All analyses were performed using IBM SPSS Statistics software version 27 (IBM Corp., Armonk, USA). The graphs were made with Origin 2016 (OriginLab Corp., Northampton, USA) and GraphPad Prism version 10.4.1 (GraphPad Software, Boston, USA).

3. Results

3.1. Total and long cTnT concentrations and their ratio in a healthy population

The characteristics of the study population are described in Table 1. Of the 314 apparently healthy individuals in the study population 59 % were female. The study population had a near-even age distribution from 21 to 85 years (Supplementary Fig. 1).

The total and long cTnT assays were able to detect concentrations above the LoD in 80 % and 98 % of individuals of the healthy population, respectively. The long cTnT assay detected concentrations above the LoD in a higher portion of males and females (100 % and 97 %, respectively) than the total cTnT assay (95 % and 69 %, respectively). The distributions of total and long cTnT concentrations were right-skewed (Fig. 2). The limited sensitivity of the total cTnT assay resulted in a high frequency peak at the 3 ng/l LoD concentration (Fig. 2A). Thus, normal distribution was not achieved with total cTnT concentrations even with a log-transformation, while the log-transformed long cTnT concentrations followed normal distribution (Supplementary Fig. 2).

The total and long cTnT concentrations and the troponin ratio, i.e. the ratio of long and total cTnT forms, in the healthy population are shown in Table 2. Both the total and long cTnT concentrations were significantly lower in females than males ($p < 0.001$ for both). However, the distributions of long cTnT concentrations in females and males overlapped considerably and were similar in shape (Fig. 3). By contrast, the distributions of total cTnT concentrations of different sexes overlapped less and had different shapes (Fig. 3). The troponin ratio was significantly higher in females than males ($p = 0.003$).

Table 1
Characteristics of the study population.

		All	Men	Women
N		314 (100 %)	130 (41 %)	184 (59 %)
Age	< 55	180 (57 %)	69 (53 %)	111 (60 %)
	≥ 55	134 (43 %)	61 (47 %)	73 (40 %)
History of smoking		102 (32 %)	49 (38 %)	53 (29 %)
Alcohol consumption	Few times a year	104 (33 %)	30 (23 %)	74 (40 %)
	Monthly	130 (41 %)	53 (41 %)	77 (42 %)
	Weekly	52 (17 %)	36 (28 %)	16 (9 %)
Medical history	Hypertension	54 (17 %)	30 (23 %)	24 (13 %)
	Dyslipidemia	55 (18 %)	30 (23 %)	25 (14 %)
	Diabetes	13 (4 %)	8 (6 %)	5 (3 %)
	Sleep apnea	17 (5 %)	8 (6 %)	9 (5 %)
	Chronic IBD	6 (2 %)	0 (0 %)	6 (3 %)
	Rheumatoid arthritis	2 (1 %)	0 (0 %)	2 (1 %)
Medication	Antithrombotic treatment	11 (4 %)	8 (6 %)	3 (2 %)
	Anticoagulant treatment	1 (0 %)	1 (1 %)	0 (0 %)
	ACE or AT2 blocker	50 (16 %)	29 (22 %)	21 (11 %)
	Statins	50 (16 %)	29 (22 %)	21 (11 %)
	Insulin	3 (1 %)	1 (1 %)	2 (1 %)
	Oral diabetes medication	8 (3 %)	5 (4 %)	3 (2 %)
	Other	108 (34 %)	37 (28 %)	71 (39 %)
	None	165 (53 %)	70 (54 %)	95 (52 %)
Heavy exercise during two days prior to sampling		6 (2 %)	5 (4 %)	1 (1 %)

Data represented as counts (percentages). Abbreviations: IBD inflammatory bowel disease, ACE angiotensin-converting enzyme inhibitor, AT2 angiotensin receptor blocker.

3.2. The 99th percentile URL

The 99th percentile URLs for the total and long cTnT assays are shown in Table 2. The imprecision of the long cTnT assay at the 99th percentile URL concentration was 5.1 % CV. The 99th percentile URL [95 % CI] of the long cTnT assay was 7.3 [5.6–8.4] ng/l for the whole reference population and higher for males (8.2 [7.0–8.2] ng/l) than for females (5.1 [4.8–5.2] ng/l).

3.3. Factors associated with the cTnT concentrations in the reference population

In individuals under the age of 55 years, the total and long cTnT concentrations had a similar weak correlation with age ($r = 0.206$ and $r = 0.234$, respectively, $p < 0.01$ for both). After the age of 55 years, the total cTnT concentration of a healthy population was heavily dependent on age ($r = 0.649$, $p < 0.01$), while the long cTnT concentration did not significantly correlate with age ($r = 0.155$, $p > 0.05$). There was a clear increasing trend in total cTnT concentrations with age after the age of 55 years, while the long cTnT concentrations remained consistently low (Fig. 4).

Common comorbidities and medications were significantly associated with the total cTnT concentration, while having only a negligible association with the long cTnT concentration of a healthy population (Table 3). Notably, none of the medications reported by the study population were significantly associated with the long cTnT concentration. By contrast, the total cTnT concentrations of individuals using medication significantly differed from the rest of the population. Similarly, individuals with hypertension and diabetes differed from the rest of the reference population regarding total cTnT concentration but not

Table 2
Total and long cTnT concentrations and the troponin ratio in the healthy reference population.

	Group	Total cTnT (ng/l)	Long cTnT (ng/l)	Troponin ratio[a] (%)
Median (25th-75th percentile)	Whole population (n = 314)	4.7 (3.3–7.3)	2.0 (1.3–3.0)	37 (24–61)
	Males (n = 130)	7.1 (4.6–9.5)	2.5 (1.7–3.6)	32 (21–54)
	Females (n = 184)	3.8 (3.0–5.2)	1.8 (1.2–2.5)	40 (26–65)
99th percentile URL[b] (CI)	Whole population	21.1 (17.5–27.1)* (18.2–24.5)**	7.3 (5.6–8.4)* (5.6–8.2)**	
	Males	26.6 (23.5–26.6)* (24.7–26.6)**	8.2 (7.0–8.2)* (7.4–8.2)**	
	Females	11.0 (10.7–11.5)* (10.8–11.0)**	5.1 (4.8–5.2)* (4.8–5.1)**	

URL upper reference limit, CI confidence interval.
[a] Troponin ratio calculated by dividing the long cTnT concentration by the total cTnT concentration
[b] Separate outlier exclusion performed for each group with the Dixon-Reed method.
* CI 95%.
** CI 90%.

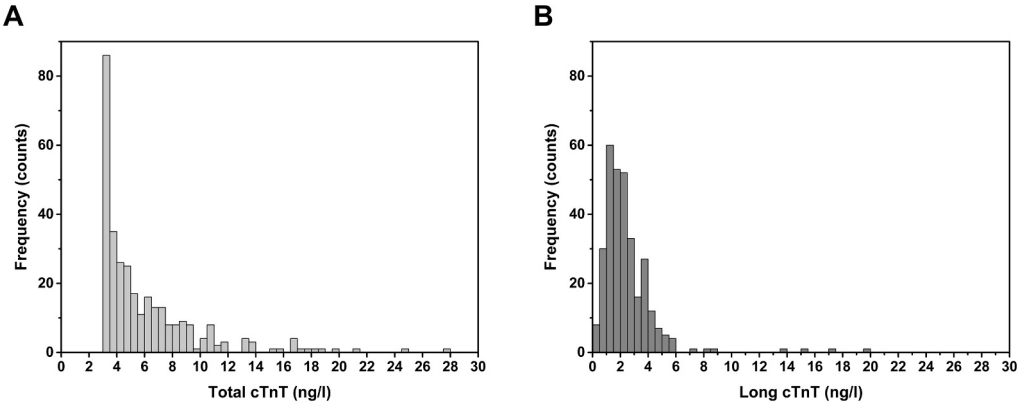

Fig. 2. Frequency histograms of the total (A) and long (B) cTnT concentrations of a healthy population.

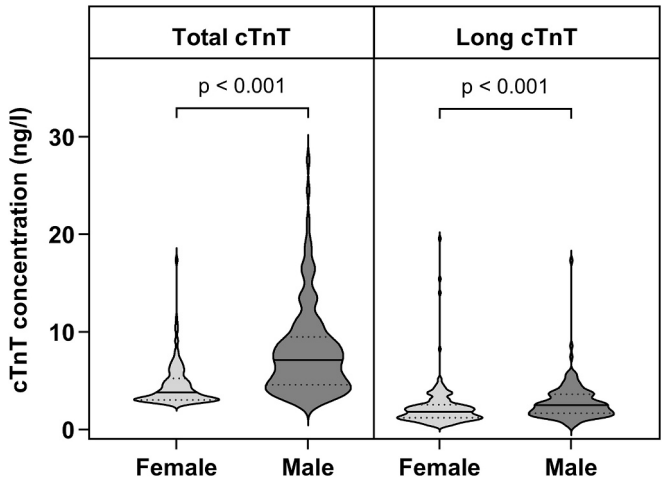

Fig. 3. Total and long cTnT concentrations in females (N = 184) and males (N = 130).

with long cTnT.

4. Discussion

The establishment of the 99th percentile URL of a healthy population is of crucial diagnostic importance for hs-cTnT assays because it is the threshold concentration used in the diagnosis of MI [1]. The influence of several factors, such as age, sex and comorbidities, on the 99th percentile URL of hs-cTnT assays is well documented [20–24]. The current study determined the concentration of long cTnT forms in a healthy reference population and established the 99th percentile URL for the novel long cTnT assay.

The results of this study are aligned with the well documented observation that sex and age significantly affect the total cTnT concentration in healthy individuals [20–24]. Importantly, in healthy individuals above the age of 55 years, the long cTnT concentration remains consistently low and does not correlate with age, while the total cTnT concentration shows a clear upward trend and significant correlation with age. Non-MI patients admitted to hospital due to a suspected cardiovascular event frequently present a total cTnT concentration above the 99th percentile URL at the emergency department [2]. This diminishes the usefulness of the clinical decision limit in the older population where MI is most prevalent. As the long cTnT concentration of a healthy population is not greatly influenced by age, the 99th percentile URL of the long cTnT assay would be better suited for universal use regardless of age.

This study confirms that our long cTnT assay fulfills the criteria of hs-cTnT assays [16–18], which are along with high sensitivity cardiac troponin I assays, the preferred assays for measuring cardiac troponins for the diagnosis of MI [16]. In comparison, previous studies have reported that the Elecsys Troponin T hs assay detects 35–55 % of a healthy study population above the LoD of 3 ng/l in lithium-heparin plasma samples, and is thus not always able to meet the criteria for high sensitivity assays [20,22,23]. In our study, a total cTnT concentration above the LoD was detected in a higher portion (80 %) of a healthy study population than previously reported, which could be explained by less stringent exclusion criteria used in our study. Regardless, there was considerable overlap with the Roche hs-cTnT 99th percentile URLs and confidence intervals reported here and in previous studies [20–23].

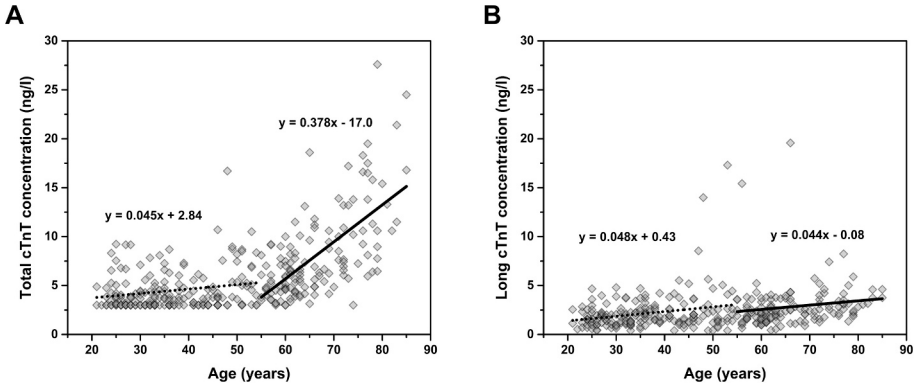

Fig. 4. The relationship between age and total (A) or long (B) cTnT concentration. Linear regression is shown separately for individuals under 55 years of age (dotted line) and for individuals aged 55 years and older (solid line).

Table 3
Total and long cTnT concentrations in different medical conditions and in groups using medications.

Medical condition	N	Total cTnT (ng/l) with	without	p-value	Long cTnT (ng/l) with	without	p-value
Hypertension	54	8.2 (4.7–11.4)	4.4 (3.1–6.4)	< 0.001*	2.3 (1.5–3.0)	2.0 (1.3–2.9)	0.158
Dyslipidemia	55	7.3 (5.4–10.6)	4.4 (3.1–6.5)	< 0.001*	2.3 (1.8–3.2)	1.9 (1.3–2.8)	0.040*
Diabetes	13	8.4 (5.4–11.2)	4.6 (3.2–7.0)	0.004*	2.2 (1.5–3.0)	2.0 (1.3–3.0)	0.588
Sleep apnea	17	7.0 (3.7–10.1)	4.6 (3.3–7.1)	0.052	2.1 (1.6–2.7)	2.0 (1.3–3.0)	0.538
Chronic IBD	6	3.5 (3.3–9.6)	4.8 (3.3–7.3)	0.634	2.0 (1.3–2.8)	2.0 (1.3–3.0)	0.852
Medication							
Antithrombotic medication	11	9.0 (6.4–17.5)	4.6 (3.3–7.1)	0.003*	2.6 (1.4–3.8)	2.0 (1.3–3.0)	0.268
ACE or AT2 blocker	50	7.8 (5.6–11.0)	4.4 (3.1–6.5)	< 0.001*	2.2 (1.5–3.1)	2.0 (1.3–2.8)	0.250
Statins	50	7.1 (5.7–10.8)	4.4 (3.1–6.7)	< 0.001*	2.2 (1.7–3.2)	2.0 (1.3–2.8)	0.086
Oral diabetes medication	8	9.1 (7.6–14.9)	4.6 (3.3–7.0)	0.001*	2.5 (2.1–3.6)	2.0 (1.3–3.0)	0.143
Other medication	108	5.5 (3.7–8.1)	4.4 (3.1–6.7)	0.008*	2.2 (1.3–3.4)	2.0 (1.3–2.8)	0.281
Any medication	165	6.0 (3.7–8.6)	4.2 (3.0–5.8)	< 0.001*	2.2 (1.4–3.3)	2.0 (1.3–2.7)	0.231

Data represented as median (25th −75th percentile).
* Statistically significant difference between groups.

An important finding of this study was that, while the total cTnT concentration is heavily influenced by common comorbidities and medications, these factors have no clear effect on the long cTnT concentration of a healthy population. This supports the inclusion of these individuals in the healthy reference population for the determination of the 99th percentile URL for the long cTnT assay. It also implies that long cTnT could be better suited for MI diagnostics than total cTnT, as patients with suspected MI are typically elderly with comorbidities.

The troponin ratio has previously been shown to be better than the total cTnT assay at discriminating between MI and other conditions characterized by elevated total cTnT [8,9,14,15]. High ratios have been typically seen in MI and lower ratios in other groups. However, the low concentrations of both total and long cTnT forms in healthy subjects in the current study resulted in relatively high troponin ratios similar to those previously reported for MI patients [8,9,14,15]. Therefore, the absolute concentrations of long and total cTnT should be considered along with their ratio to avoid misinterpretation. Nevertheless, the troponin ratio can have additional diagnostic value as it reflects the composition of circulating cTnT forms.

There are limitations to this study that need to be considered. Firstly, the health status of the study cohort was based solely on a health questionnaire and thus unknown or non-disclosed conditions could affect the results. Very strict exclusion criteria based on complementary laboratory tests have been provided by international guidelines to help define a healthy reference population for hs-cTnT assays [16,17]. However, our finding that common comorbidities and medications showed little impact on the long cTnT concentration is clinically important and implies that more inclusive criteria for a healthy reference group could be allowed for long cTnT assays. Secondly, a minimum of 800 subjects, including 400 males and females, is recommended to establish separate cut-off concentrations for the whole population and each sex [16]. Because these criteria were not met in the present study, the 99th percentile URLs presented here should be considered as estimates.

The ability of the long cTnT assay to discriminate between MI and other conditions using the 99th percentile URL established in the current study should be evaluated in a clinical setting to validate its diagnostic performance.

In conclusion, we determined the long cTnT concentrations in a healthy reference population, established the 99th percentile URL for our long cTnT assay, confirmed that the assay meets the criteria for hs-cTnT assays and evaluated correlations with age, sex and comorbidities. The results presented in this study can be used as a basis for further research on the diagnostic performance of long cTnT forms.

CRediT authorship contribution statement

Tuulia Tuominen: Writing – original draft, Visualization, Validation, Investigation, Formal analysis, Data curation. **Tuija Vasankari:** Writing – review & editing, Supervision, Project administration, Investigation, Data curation, Conceptualization. **Helea Junes:** Writing – review & editing, Investigation. **Selma Salonen:** Writing – review & editing, Investigation. **Konsta Teppo:** Writing – review & editing, Investigation, Conceptualization. **Anna Linko-Parvinen:** Writing – review & editing, Resources, Conceptualization. **Hanna-Mari Pallari:** Writing – review & editing, Resources, Conceptualization. **K.E. Juhani Airaksinen:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Saara Wittfooth:** Writing – review & editing, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization.

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Declaration of competing interest

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cca.2025.120419.

Data availability

The data that has been used is confidential.

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