

RESEARCH LETTER

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Comparing baseline and reaction samples of perioperative anaphylaxis patients reveals IL-6 and CCL2 as potential biomarkers

Anaphylaxis is the most severe manifestation of allergy, often with rapid, unexpected onset, leading to the development of life-threatening symptoms from airway and/or circulation. Despite many years of research, the specific mechanisms involved and biomarkers for detecting anaphylaxis are still elusive.^{1,2}

Presently, tryptase is the only biomarker used routinely in the diagnosis of anaphylaxis. Its level is stable within individuals but varies within the population. Therefore, measurements of tryptase to support diagnosis of anaphylaxis are determined by comparing tryptase levels in serum samples from the reaction with the patient's own baseline sample.² Other potential biomarkers have been reported in anaphylaxis patients, either measured during reaction or in baseline samples. However, comparison between reaction and baseline samples in the same patient is rarely available. Therefore, we investigated nine biomarkers with potential involvement during anaphylaxis in reaction and baseline samples from a well-characterized group of patients ($n = 13$) with perioperative anaphylaxis (grade 3–4 on Ring and Messmer scale, Table S1). The patients all had elevated tryptase at the time of reaction and were subsequently investigated at the Danish Anaesthesia Allergy Center where a causal agent was identified. Surgical controls included 3 patients referred to the Danish Anaesthesia Allergy Center with suspected perioperative anaphylaxis due to severe refractory perioperative hypotension. These showed no tryptase elevation, had negative investigations, and hypotension was attributed to treatment with tricyclic antidepressants.³ Furthermore, serum samples from 4 patients with grass pollen allergy were included, which together with the baseline samples of the surgical control patients, made up the baseline control group. Clinical patient data are shown in Table 1.

The selection of biomarkers was either based on previous reports illustrating their presence in anaphylaxis patients (baseline and/or reaction samples) or linked to a cellular source (i.e., platelets or basophils) involved in the anaphylactic reaction. The outline for selecting each analyte is found in Table S2. All serum samples included were tested for all nine biomarkers on the same plate using ProcartaPlex Multiplex Immunoassay as described by the manufacturer (see for further details).

Of the nine biomarkers tested, IL-10, tumour necrosis factor receptor 1 (TNFRI) and CCL2 were significantly up-regulated ($p = .027$,

.008 and .033, respectively) during perioperative anaphylaxis (anaphylaxis reaction) compared with the anaphylactic patient's own baseline sample (anaphylaxis baseline) (Figure 1A). IL-6 was also elevated in anaphylaxis reaction samples but did not reach significance level ($p = .068$, Figure 1A). These findings are in agreement with other studies reporting an increase in IL-6, IL-10, TNFRI and CCL2 in patients with anaphylaxis to different elicitors.^{4–6} However, when analysing the surgical control patients, we saw a tendency of an increased level of IL-6, IL-10 and TNFRI following the hypotensive response in these patients during surgery despite the non-allergic mechanism (Table S3). In contrast, CCL2 seemed less affected by the surgical setting, giving it the potential as a more specific biomarker of perioperative anaphylaxis.

The five other biomarkers tested (CCL3, vascular endothelial growth factor A (VEGF-A), TNF-like weak inducer of apoptosis (TWEAK), CD40L and CXCL12) showed no significant changes during anaphylaxis (Figure 1A). TWEAK has previously been reported to be elevated in plasma of patients during anaphylaxis compared with healthy controls.⁷ We could not confirm this observation, neither when comparing with patient's own control (anaphylaxis baseline) nor the baseline control group (data not shown).

Interestingly, when comparing anaphylaxis baseline samples with the control group, a significant difference was found for IL-6 ($p = .030$) indicating that anaphylactic patients may have an elevated baseline level of IL-6 (Figure 1B). These baseline levels were measured in samples taken at least two weeks (in most cases several months) after the anaphylactic episode and were therefore not caused by an increase in IL-6 during anaphylaxis. A previous study reported similar levels of IL-6 in baseline samples of children with recurrent anaphylaxis compared with a control group.⁸ If this finding could be confirmed in other populations, it might have the potential to help identify patients at risk of anaphylaxis. Admittedly, numbers of patients and controls are small, but the paired analysis is a strength of this study. Further investigations in other populations are needed to confirm our findings.

We did not detect a significant increase in IL-6 during anaphylaxis as reported by others who have compared reaction samples with baseline samples of a control group instead of with the patient's own baseline level.^{4,6} This highlights the importance of including the

patient's own baseline sample as the reference sample as is already standard practice for tryptase. Reduced IL-10 level has previously been found in baseline samples from patients who have experienced food-related anaphylaxis,⁹ and low baseline levels of CCL2 and CCL3, as well as an elevated level of VEGF-A, have been shown in serum samples from children with recurrent anaphylaxis to cow's milk.⁸ We did not find any of these differences in the included perioperative anaphylaxis patients (Figure 1B). Further investigations into baseline samples of specified groups of anaphylaxis patients could help explain these differences.

Finally, Spearman correlation was made for all biomarkers in anaphylaxis baseline and reaction samples. Here, we found that in the reaction samples, both IL-10 and TNFRI correlated with IL-6 and CCL2, but not with each other (Table S4), illustrating that all the elevated biomarkers were not exclusively from the same patients. Surprisingly, none of the elevated biomarkers were found

Key Messages

- CCL2 serum levels increase during perioperative anaphylaxis and may be a valuable supplement to tryptase measurement.
- IL-6 was generally increased at baseline in patients with perioperative anaphylaxis compared with control group.
- The patient's own baseline sample is important when investigating biomarkers related to anaphylaxis.

to correlate with tryptase. An explanation could be that IL-6, IL-10 and TNFRI seemed more linked to the surgical setting rather than anaphylaxis and therefore would not follow tryptase. In contrast, CCL2 did stand out, together with tryptase, as the only elevated

TABLE 1 Demographic and clinical data of included patients

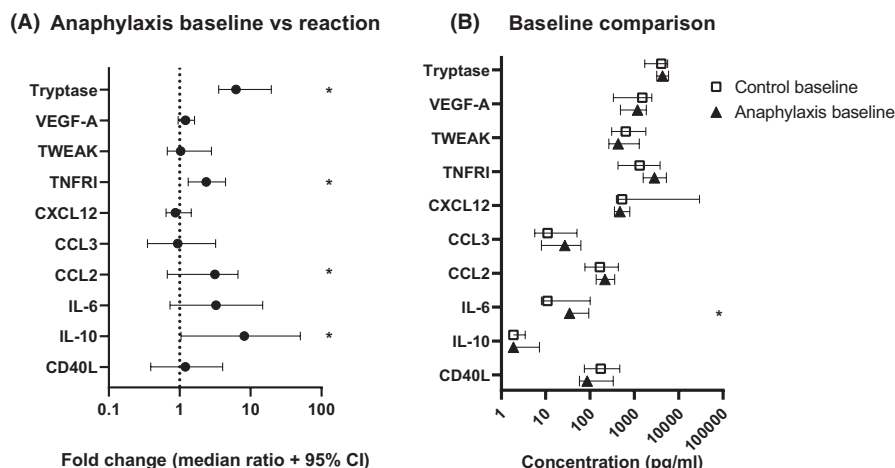
Patient ID	Age at reaction (y) ^a	Sex	Ring and Messmer reaction grade	Culprit drug	Time from culprit exposure to reaction (min)	Reaction onset to blood sampling (min)	Reaction tryptase (µg/l)	Baseline tryptase (µg/l)	Other reported allergies/sensitization to inhalant allergens
AX1	60	F	3	Cefuroxime (i.v.)	2	180	48.5	3.3	Sulphamethizole
AX2	43	F	3	Cefuroxime (i.v.)	5	200	18.3	3.74	Cat, grass, birch
AX3	63	F	3	Cefuroxime (i.v.)	10	75	102	4.4	–
AX4	60	F	3	Ampicillin (i.v.)	6	45	41.7	5.91	–
AX5	63	F	3	Tranexamic acid (i.v.)	5	95	22.4	4.97	–
AX6	59	F	3	Penicillin G (i.v.)	15	60	19.9	6.79	–
AX7	48	F	3	Piperacillin/Tazobactam (i.v.)	20	20	21.1	6.05	–
AX8	73	F	3	Morphine (i.v.)	10	60	23.9	3.21	–
AX9	55	M	3	Cefuroxime (i.v.)	2	90	19.6	<1	–
AX10	41	M	3	Rocuronium (i.v.)	2	110	26.9	4.32	–
AX11	19	M	3	Rocuronium (i.v.)	2	105	10.7	2.08	Birch, grass, dog, cat, moulds ^b
AX12	27	F	3	Mannitol (i.v.) Excipient in i.v. paracetamol	5	80	18.7	5.28	House dust mites
AX13	75	F	4	Dicloxacillin (i.v.)	5	245	76.6	3.65	–
SC1	68	M	3	Allergy disproved	–	55	4.42	4.94	–
SC2	63	M	3	Allergy disproved	–	180	4.6	4.05	–
SC3	61	F	3	Allergy disproved	–	Unknown	5.18	5.62	Pivampicillin
AC1	56	M	–	–	–	–	–	3.42	Grass
AC2	49	F	–	–	–	–	–	4.07	Grass
AC3	43	F	–	–	–	–	–	1.71	Grass
AC4	25	F	–	–	–	–	–	2.29	Grass

Abbreviations: AC, allergic control; AX, anaphylaxis; SC, surgical control.

^aFor AC1-4; Age at allergy test.

^bCladosporium & Alternaria.

FIGURE 1 Comparison of biomarker levels in serum samples. (A) Median ratio and 95% confidence interval (CI) for fold change in baseline and reaction samples of anaphylaxis patients ($n = 13$). (B) Comparison of biomarker level in baseline samples of control group (squares, $n = 7$) and anaphylaxis patients (triangles, $n = 13$). * $p < .05$



biomarkers during anaphylaxis. However, whereas tryptase mainly originated from mast cells, several cells (e.g., monocytes, macrophages, dendritic cells and endothelial cells) could be responsible for the release of CCL2. Furthermore, the dynamic in release and uptake might differ between the two biomarkers.

In anaphylaxis baseline samples, correlation was found between IL-6 and IL-10 and, surprisingly, CD40L and TWEAK, as well as CD40L and CCL3 (Table S4). This correlation was found to be due to a small group of patients with elevated CD40L and TWEAK (and partly CCL3) levels (Figure S1). This difference could not be linked to any clinical observations.

A weakness of our study is the limited number of subjects, and it is clear that a higher number might have strengthened our findings. Because of this, age and gender distribution are also not as diverse as could be wished for. Well-characterized patients are rare, however, and we gave priority to strict inclusion criteria to get a clearer picture. It could also be argued that the use of historical samples may introduce analytical problems. Whereas this cannot be totally excluded, we did not for any analyte see a relation between the age of samples and the quantitation (data not shown).

In conclusion, out of the nine assessed biomarkers, we found two potential biomarkers associated with anaphylaxis in a well-characterized group of perioperative anaphylaxis patients. CCL2 was found to be elevated during anaphylaxis and less affected by the surgical setting than the other elevated biomarkers. IL-6 was found to be higher in the baseline samples of perioperative patients compared with the control group.

KEYWORDS

Anaphylaxis, CCL2, IL-6, perioperative allergy, serum tryptase

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CONFLICT OF INTEREST

All authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

KTC, LKP, LHG and BMJ designed the project, KTC carried out the experimental work, LHG recruited and characterized the human subjects, KTC wrote the initial version of the manuscript and all four contributed to the final version of the manuscript.

DATA AVAILABILITY STATEMENT

Research data are not shared.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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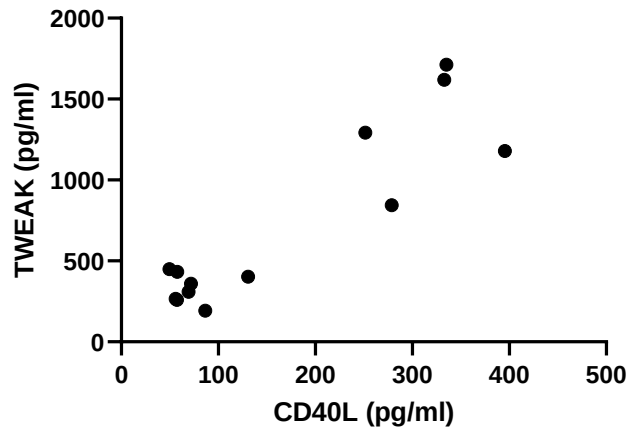
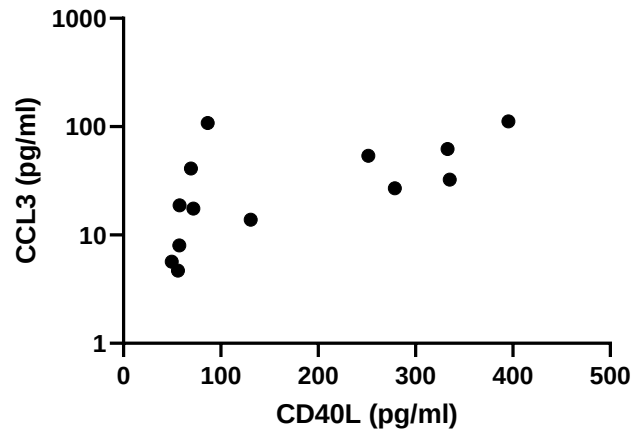
A**B**

Figure S1 – Correlation between CD40L and TWEAK (A) or CCL3 (B) in anaphylaxis baseline serum samples.

Table S1 – Ring and Messmer grading scale (modified from ref^{1,2})

Grade	Symptoms
I	Skin and mucosal signs only
II	Moderate signs from several organs
III	Severe, life-threatening signs from one or more organs
IV	Circulatory and/or respiratory arrest

Table S2 – Reasoning behind choosing the specific analytes for testing.

Analyte	Function	Reason for interest
CD40L	Proinflammatory cytokine from the tumor necrosis factor family.	Exists both as cell-bound and soluble forms. Mainly released by platelets. Suggested serum levels could be used to indicate total platelet pool ³ .
IL-6	Proinflammatory cytokine	Shown to be upregulated in patients with anaphylaxis to different elicitors from emergency departments ^{4–6} . Baseline levels shown to be unchanged in children with recurring anaphylaxis to milk ⁷ .
IL-10	Anti-inflammatory cytokine	Shown to be upregulated in patients with anaphylaxis to different elicitors from emergency departments ^{4–6} . Baseline levels shown to be unchanged in children with recurring anaphylaxis to milk ⁷ but decreased in other food anaphylaxis group ⁸ .
CCL2 (Monocyte chemotactic protein-1, MCP-1)	Chemokine for monocytes	Shown to be upregulated in venom anaphylaxis (and minor peanut reaction to oral challenge) ⁹ . Baseline levels shown to be decreased in children with recurring anaphylaxis to milk ⁷ .
CCL3 (Macrophage inflammatory protein-1α, MIP-1α)	Pro-inflammatory chemokine	Baseline levels shown to be decreased in children with recurring anaphylaxis to milk ⁷ .
CXCL12 (Stromal cell–derived factor-1α, SDF-1α)	Chemokine for lymphocytes and basophils.	Might be important for recruitment and activation of basophils ¹⁰ .
TWEAK (TNF-like weak inducer of apoptosis)	Implicated in proliferation, migration, angiogenesis, apoptosis, and inflammation	Shown to be upregulated in patients with anaphylaxis to different elicitors from emergency department ¹¹ .
TNFR1	One of two TNF- α receptors.	Exists both as cell-bound and soluble forms. Soluble form surrogate marker for TNF- α ¹² . Shown to be upregulated in patients with anaphylaxis to different elicitors from emergency departments ^{4,5} .
VEGF-A (Vascular Endothelial Growth Factor A)	Essential for angiogenesis.	Baseline levels shown to be increased in children with recurring anaphylaxis to milk ⁷ .

Table S3 – Concentration of IL-10, IL-6, CCL2 and TNFRI in baseline and “reaction” samples of surgical control patients.

	IL-10 (pg/ml)			IL-6 (pg/ml)			CCL2 (pg/ml)			TNFRI (pg/ml)		
	Baseline	Reaction	Fold change	Baseline	Reaction	Fold change	Baseline	Reaction	Fold change	Baseline	Reaction	Fold change
SC1	1,87*	4,43	2,37	13,99	48,19	3,44	76,97	66,58	0,87	428,76	1875,42	4,37
SC2	1,87*	1,87*	1,00	10,93	21,97	2,01	205,92	282,36	1,37	1140,47	1722,77	1,51
SC3	1,87*	1,99	1,06	28,53	31,03	1,09	110,69	107,41	0,97	3347,78	6943,9	2,07

*Reference value for lower limit of detection of assay as true value was below. Fold change: Reaction value/baseline value

Table S4 – Correlation between biomarkers in either anaphylaxis baseline (blue) or anaphylaxis reaction (orange) serum samples.

	Tryptase	CD40L	IL-10	IL-6	CCL2	CCL3	CXCL12	TNFR1	TWEAK	VEGF-A	
Tryptase	1	-0.1319	-0.1566	0.0714	-0.4286	0.0550	0.1374	0.1758	-0.0110	-0.1484	Anaphylaxis baseline
CD40L	-0.0714	1	0.0846	-0.2198	-0.0604	0.7527	0.2967	0.3791	0.6758	0.2802	
IL-10	0.3626	-0.0714	1	0.7110	0.0814	0.0533	0.3727	0.4291	-0.0376	-0.0376	
IL-6	0.0275	0.1868	0.7912	1	-0.1374	-0.1154	0.4725	0.3791	-0.2033	-0.1209	
CCL2	0.0769	0.0275	0.5879	0.5220	1	0.1813	0.1868	0.1154	-0.4835	0.2912	
CCL3	0.000	-0.0110	0.3736	0.4835	0.4121	1	0.5110	0.4615	0.3626	0.4176	
CXCL12	-0.1484	0.3022	-0.0385	0.1429	-0.1923	-0.0495	1	0.2692	-0.0714	0.2033	
TNFR1	-0.1264	0.4945	0.2912	0.6044	0.6593	0.4176	0.0879	1	0.1538	0.0824	
TWEAK	-0.1044	0.4231	0.3516	0.4451	0.3681	0.4231	-0.0659	0.3187	1	-0.1538	
VEGF-A	-0.3297	0.2198	-0.0934	0.2527	0.1538	0.0165	0.3297	0.5440	-0.2857	1	
Anaphylaxis reaction											

Spearman correlation coefficient (rho) is depicted. Rho with p-val<0.05 is highlighted in bold.

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Supporting information

Materials and methods

Participants

Thirteen patients that had been referred to and fully investigated in the Danish Anaesthesia Allergy Center following perioperative anaphylaxis were retrospectively included. Inclusion criteria were (i) anaphylaxis during surgery to an intravenous culprit, (ii) at least a grade 3 severity score reaction (Ring and Messmer scale, **Table S1**), (iii) a baseline tryptase <11.4 ng/ml, (iv) an elevated tryptase level in reaction sample based on the algorithm [reaction tryptase $\geq 1.2 \times$ baseline tryptase + 2 ng/ml], and (v) the culprit was identified by two or more of the following methods: sIgE measurements, skin prick test, intradermal test, histamine release tests or provocation tests.

As controls two smaller groups were included; 1) four patients (allergic controls) that were highly positive on specific IgE to grass (4.57-75 PRU/ml), but other inhalation allergens tested negative (house dust mites, cat, dog, horse, molds, birch and mugwort), and 2) three patients (surgical controls) that were investigated at the Danish Anaesthesia Allergy Center following perioperative hypotension suspected to be due to anaphylaxis, but where all investigations were negative including elevated serum tryptase at the time of reaction, and treatment with tricyclic antidepressants was concluded to contribute to the perioperative hypotension.

Serum samples

This retrospective study was approved by the Ethical Committee of the Danish Capital Region and the relevant Data Authority (file number H-18016720; data management approval number VD-2018-502). All samples were taken between 2012-2017 and stored at -20°C until use. From all anaphylactic patients and surgical control patients, two serum samples were used; one taken at the time of reaction during surgery ("reaction sample") and one taken at least two weeks after total resolution of all signs and symptoms ("baseline sample"). Only one sample was used from the allergic control patients. This was taken outside grass pollen season ("baseline sample").

Biomarker assays

Tryptase was measured using Phadia ImmunoCAP (Thermo Fisher Diagnostics, Uppsala, Sweden). The analysis has a limit of detection of 1 µg/l according to the manufacturer.

All other biomarkers were measured simultaneously and in duplicates using ProcartaPlex Multiplex Immunoassay, Mix&Match panels, 9-plex (Invitrogen, Thermo Fisher Scientific, Slangerup, DK) according to the manufacturer's instructions. The samples were analyzed on a Bio-Plex 200 system with Bio-Plex Manager 5.0 (BioRad, Berkeley, CA, USA). Limit of detection for each biomarker was according to the manufacturer as follows; CD40L: 3.25 pg/ml, IL-6: 8.01 pg/ml, IL-10: 1.87 pg/ml, CCL2: 3.56 pg/ml, CCL3: 1.87 pg/ml, CXCL12: 16 pg/ml, TWEAK: 37 pg/ml, TNFRI: 189 pg/ml, and VEGF-A: 5.37 pg/ml.

Statistics

Wilcoxon matched-pairs signed rank test was used to compare difference between anaphylaxis baseline and reaction samples while the Mann Whitney test was used to compare anaphylaxis baseline samples to control baseline samples (combined surgical and allergic control samples). Illustration of anaphylaxis baseline and reaction sample comparison was made as fold change (reaction/baseline level) while measured concentrations were used in the illustration of anaphylaxis baseline levels compared to control group baseline. Both illustrations are made as median + 95% confidence interval. All statistical analysis and data illustrations were performed using Graphpad Prism 8.3.0.