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Cytokine architecture in gadolinium deposition disease: integrated analysis of baseline, post-chelation, and dynamic immune responses

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Abstract

Background: Gadolinium Deposition Disease (GDD) is a chronic, persistent illness arising in susceptible individuals after administration of a gadolinium-based contrast agent (GBCA). Three previous human studies evaluated serum cytokines in GDD at baseline, approximately 24 hours after Ca-DTPA chelation, and during the first minutes through 24 hours after chelation.

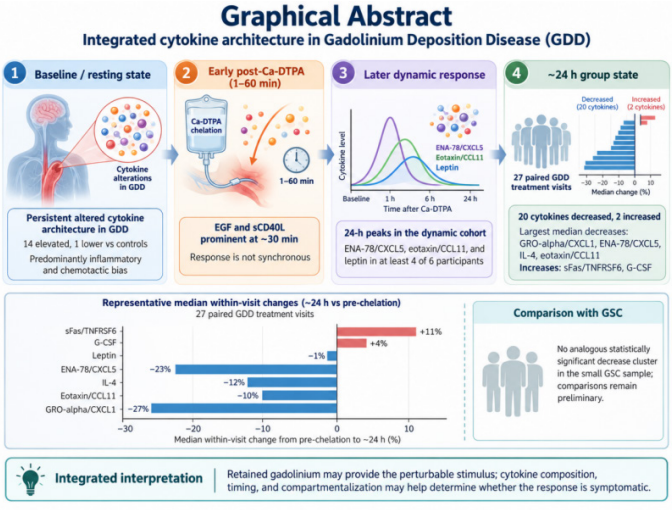
Purpose: To synthesize the findings of three previously published human cytokine studies, together with a descriptive reanalysis of original paired data, into a cohesive temporal and functional model, and determine whether GDD is characterized by a cytokine architecture involving resting-state dysregulation, temporally staggered responses to gadolinium mobilization, and a distinct 24-hour post-chelation pattern.

Materials and methods: This secondary analysis integrated three previously published multiplex Luminex studies: 24 GDD participants versus 64 healthy controls; 21 unique GDD and 3 unique asymptomatic Gadolinium Storage Condition (GSC) subjects studied before and approximately 24 hours after Ca-DTPA, including repeat visits; and a dynamic cohort of 4 GDD and 2 GSC subjects sampled at T0, 1, 5, 10, 30, and 60 minutes and 24 hours. Cytokines were interpreted in overlapping functional domains. For the second study, original raw MFI data and contemporaneous sample keys were descriptively reanalyzed across 27 paired GDD treatment visits.

Results: At baseline, the first study identified 14 elevated cytokines and one lower cytokine in GDD versus controls, with a predominant inflammatory and chemotactic bias. The second study identified 22 cytokines that changed significantly from pre-chelation to approximately 24 hours in GDD: 20 decreased and sFas/TNFRSF6 and G-CSF increased. None of these 22 showed a statistically significant decrease in the small GSC comparison group; CTACK/CCL27 increased in GSC. Raw-data descriptive reanalysis of 27 GDD visits reproduced the direction of all 22 changes; median changes included GRO-alpha/CXCL1 -26.6%, ENA-78/CXCL5 -23.4%, IL-4 -11.7%, eotaxin/CCL11 -10.2%, sFas/TNFRSF6 +11.3%, and G-CSF +3.9%. In the dynamic study, EGF and sCD40L peaked at approximately 30 minutes in at least 4 of 6 subjects, whereas ENA-78/CXCL5, eotaxin/CCL11, and leptin peaked at 24 hours in at least 4 of 6, demonstrating a staggered response. One GDD and one asymptomatic GSC subject were broad cytokine high-outliers despite different symptom phenotypes.

Conclusions: The combined data support a model in which GDD is associated with an altered resting cytokine state and a temporally organized response to gadolinium mobilization. The coordinated 24-hour decrease in multiple circulating cytokines in GDD, enriched for chemotactic and trafficking mediators, may reflect redistribution or compartmentalization rather than simple suppression. A large systemic cytokine response can occur without symptoms in GSC, suggesting that functional composition, timing, tissue localization, and downstream organization may be more important than absolute cytokine magnitude. GDD-GSC comparisons remain preliminary because of the small GSC sample.

Graphical abstract: Integrated interpretation of cytokine profiles in GDD: resting-state dysregulation, temporally staggered cytokine responses after Ca-DTPA, and the approximately 24-hour pattern. Based on the three previously published source studies and descriptive reanalysis reported in this manuscript.



Keywords: gadolinium, inflammatory cells, gene expression, cytokine production, biological state

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Introduction

Gadolinium Deposition Disease (GDD) is a chronic, persistent illness that arises after administration of a gadolinium-based contrast agent (GBCA) in susceptible individuals, usually in the setting of normal or near-normal renal function.[1–4] The disorder is characterized by a reproducible multisystem symptom complex beginning after GBCA exposure and by persistence of gadolinium beyond the expected period of vascular elimination. GDD fits the broader biological framework of heavy-metal toxicity: exposure to an exogenous metal, retention within biological compartments, interaction with cellular and molecular systems, and, when chemically accessible, potential removal by decorporation.[5]

An immunological contribution to GDD is biologically plausible. GBCAs have been shown experimentally to induce cytokine, chemokine, interferon, and growth-factor responses in human monocytes,[6,7] and human studies have demonstrated abnormalities in circulating cytokines in patients meeting clinical criteria for GDD.[8–10] Cytokines therefore provide a measurable window into both the resting biological state and the response to perturbation of retained gadolinium.

Independent studies provide mechanistic context for the human cytokine findings. Del Galdo and colleagues observed NF-κB activation and enhanced CC/CXC chemokine production when human macrophages were exposed to gadodiamide-containing contrast medium; Wermuth and Jimenez implicated TLR4/TLR7 pathways in gadolinium-associated macrophage activation.[11,12] Schmidt-Lauber and colleagues reported NLRP3-dependent IL-1β release in murine macrophages and recruitment of inflammatory cells following intraperitoneal administration of a gadolinium-containing compound in mice.[13] Weng and colleagues found agent- and condition-dependent changes in macrophage oxidative stress and cytokine production at comparatively low experimental GBCA concentrations.[14] These experiments demonstrate biologically credible pathways for immune perturbation; none establishes the mechanisms or prevalence of GDD in humans.

Additional cellular models suggest that gadolinium chemistry and intracellular handling may influence the phenotype of responding cells. Gadolinium-associated calcium-phosphate precipitation was linked with profibrotic macrophage activation *in vitro*.[15] Studies using gadoterate-exposed macrophages documented retained gadolinium, changes in RhoA/mTOR-related proteins, and alterations in adhesion, motility, or immune-related gene expression; experiments combining gadoterate with magnetic-field gradients also reported macrophage morphological and polarization changes.[16–18] These findings are relevant to hypotheses about tissue-localized responses, but experimental concentrations, exposure schedules, and cell models differ materially from the clinical setting.

Three prior human studies examined complementary aspects of this biology.[8–10] The first characterized the baseline or resting serum cytokine profile of patients with GDD relative to healthy controls.[8] The second evaluated cytokines immediately before and approximately 24 hours after Ca-DTPA chelation in GDD and asymptomatic Gadolinium Storage Condition (GSC).[9] The third used serial measurements from immediately before chelation through 24 hours afterward to examine the temporal evolution of the response.[10]

The principal contribution of this work is a synthesis of three separately published human cytokine investigations into a unified, biologically interpretable account of cytokine behavior before and after Ca-DTPA chelation. Rather than treating the source reports as isolated observations, we examine their shared and differing findings across baseline, minute-scale, and approximately 24-hour measurement windows, supported by a new descriptive reanalysis of the original paired data. The purpose of the present investigation was to integrate these data as a single temporal and functional model of GDD cytokine biology. We evaluated whether GDD is associated with an altered resting cytokine architecture; whether Ca-DTPA-associated gadolinium mobilization produces a coordinated 24-hour pattern that differs from asymptomatic GSC; and whether high-temporal-resolution data indicate staggered, rather than synchronous, cytokine responses. We hypothesized that the clinically relevant distinction between GDD and GSC may depend more on functional composition, timing, tissue localization, and downstream organization of the immune response than on the absolute magnitude of circulating cytokine release.

Materials and methods

Study design

This investigation was an evidence synthesis and integrated secondary analysis of three previously published human cytokine studies of GDD.[8–10] No new intervention or specimen collection was performed for the present analysis. Original enrollment, informed consent, institutional review, clinical definitions, specimen handling, laboratory assays, and primary statistical methods are detailed in the source publications. The current analysis treated the studies as sequential biological windows: resting state, acute perturbation by Ca-DTPA-associated gadolinium mobilization, and temporal evolution of the response from minutes to approximately 24 hours.

Source studies and cytokine measurement

Study 1 included 24 GDD participants and 64 healthy controls and used a Luminex 62-plex serum cytokine assay.[8] Study 2 included 21 unique GDD subjects and 3 unique GSC subjects. Six GDD subjects and one GSC subject contributed a second treatment visit, yielding 27 paired GDD treatment visits and 4 paired GSC treatment visits in the reconstructed sample set. Serum was obtained immediately before Ca-DTPA and approximately 24 hours later, at approximately the same time of day, and 76 cytokines were measured using a three-panel Luminex assay.[9] Study 3 included 4 GDD and 2 GSC subjects and used the same general 76-plex approach, with serum sampling at T0 before Ca-DTPA and at 1, 5, 10, 30, and 60 minutes and 24 hours after treatment.[10] Median fluorescence intensity (MFI) of duplicate wells was used as the analytical signal in Studies 2 and 3, consistent with the original methodology and prior validation of raw fluorescence signals in multiplex immunoassays.[19]

Integrated data handling

Original raw assay files, contemporaneous sample-receiving lists, and patient-level pre/post keys were reviewed to reconstruct treatment-pair assignments for Study 2. Because the three studies used different assay panels, control cohorts, batches, and sampling designs, raw MFI values were not pooled across studies as a single numerical dataset. Instead, the integrated analysis preserved the original within-study comparisons and examined cross-study direction, timing, functional grouping, and response architecture. This approach avoids treating absolute fluorescence values from different experimental contexts as interchangeable.

Functional cytokine classification

For biological interpretation, cytokines were grouped according to predominant or particularly relevant functions, including inflammatory/

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innate activation, chemotaxis and leukocyte trafficking, T-cell/NK-cell signaling, endothelial/adhesion biology, regulatory/homeostatic signaling, epithelial/barrier signaling, hematopoietic signaling, and growth/repair pathways. These categories were intentionally allowed to overlap. Individual cytokines can exert different, and sometimes opposing, effects depending on responding cell type, receptor expression, concentration, timing, interacting mediators, and the local tissue microenvironment. Assignment to a functional category therefore does not define a single invariant biological role. Circulating concentration also does not necessarily reflect activity within a tissue compartment; movement of immune cells, local cytokine production, receptor binding, consumption, degradation, and clearance can all alter the relationship between serum cytokine levels and tissue-level immune activity.

Descriptive raw-data reanalysis of the 24-hour cohort

For the 22 cytokines previously shown by the original mixed-effects analysis to change significantly from pre-chelation to approximately 24 hours in GDD,[9] a new descriptive reanalysis was performed using the reconstructed raw MFI pairs from the 27 GDD treatment visits. For each analyte and visit, percent change was calculated as 100 x (post-chelation MFI - pre-chelation MFI) / pre-chelation MFI. The median percent change and the number of visits showing the direction identified in the original inferential analysis were calculated. These descriptive statistics were used to characterize effect direction and consistency; they were not used to generate new claims of statistical significance.

Original statistical analyses

Study 1 used Wilcoxon rank-sum testing for GDD-versus-control comparisons, with Benjamini-Hochberg correction for non-hypothesized cytokines as specified in the original report.[8] Study 2 used linear mixed-effects models to evaluate pre-to-post changes and whether changes differed between GDD and GSC, with patient-visit-specific intercepts; the original work was exploratory and did not apply a multiple-comparison correction.[9] Study 3 was a pilot kinetic study. A 30-minute peak was defined as a value at least 20% higher than the 10-minute value in at least 4 participants, and a 24-hour peak as a value at least 20% higher than the 60-minute value in at least 4 participants. High outliers were defined from participant-level mean cytokine values using Q3 + 3 x IQR.[10] The present manuscript reports those inferential findings as source-study results and adds the descriptive Study 2 reanalysis described above.

Results

Baseline/resting cytokine architecture

The first study demonstrated an altered resting circulating cytokine state in GDD [8]. Fourteen cytokines were significantly elevated relative to healthy controls: TNF-alpha, GRO-alpha/CXCL1, TGF-alpha, IL-15, IL-6, MCP-3/CCL7, VCAM-1, IL-18, IL-23, IL-2, SCF, IL-8/CXCL8, IL-31, and LIF. MCP-1/CCL2 was significantly lower. Nine of the elevated cytokines were classified in the original report as pro-inflammatory. Functionally, the pattern was enriched for inflammatory/innate and chemotactic signaling, while also including T-cell/NK-cell, endothelial/adhesion, and growth/homeostatic mediators. The magnitudes of the median differences were generally modest and the participant and control distributions overlapped, consistent with an altered biological state rather than a binary diagnostic biomarker.

The pre-chelation comparison in the second cohort independently demonstrated resting-state cytokine abnormalities, although the individual analyte pattern was not identical.[9] Six cytokines were higher than healthy controls (MIP-1delta/CCL15, CTACK/CCL27, TRAIL, G-CSF, GRO-alpha/CXCL1, and eotaxin-2/CCL24) and six were lower (sCD40L, PAI-1, EGF, HGF, IFN-alpha2, and IL-12p70). GRO-alpha was the only elevated cytokine shared by the two baseline studies. Thus, the combined baseline data support cytokine dysregulation in GDD but do not support a rigid, invariant single-analyte signature across cohorts Table 1 & Figure 1.

Table 1 Temporal cytokine architecture across the three source studies

Biological window	Sampling	Cohort	Principal cytokine observation	Integrated significance
Baseline/resting state	No acute Ca-DTPA challenge	24 GDD vs 64 healthy controls	14 cytokines elevated, 1 lower; predominantly inflammatory/ chemotactic bias	Establishes an altered resting cytokine state in GDD
Independent pre-chelation baseline	Immediately before Ca-DTPA	21 unique GDD; healthy controls	6 cytokines higher and 6 lower than controls; GRO-alpha shared with Study 1	Supports dysregulation, but not a rigid single-analyte signature
Early post-Ca-DTPA	T0 to 60 min	4 GDD + 2 GSC	EGF and sCD40L peaked at ~30 min in at least 4/6	Demonstrates an early phase and non-synchronous signaling
Later dynamic phase	60 min to 24 h	4 GDD + 2 GSC	ENA-78/CXCL5, eotaxin/CCL11, leptin peaked at 24 h in at least 4/6	Demonstrates staggered/late signaling
~24-h group state	Pre to ~24 h	27 paired GDD visits; 4 GSC visits	20 cytokines decreased and 2 increased in GDD; no analogous significant decrease cluster in GSC	Supports a distinct later circulating cytokine architecture in GDD

Note: GDD, gadolinium deposition disease; GSC, gadolinium storage condition; Ca-DTPA, calcium diethylenetriaminepentaacetic acid. The GSC comparison is preliminary because of the small sample.

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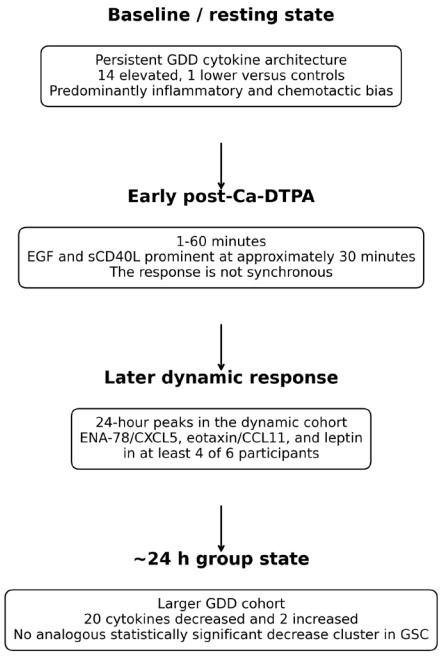


Figure 1 Integrated temporal model of cytokine architecture in GDD. The figure summarizes the baseline/resting cytokine state, early and later dynamic post-Ca-DTPA responses, and the approximately 24-hour group state. The model emphasizes that different cytokine programs may be prominent at different intervals and that the 24-hour circulating state represents a later phase of an evolving response.

Cytokine architecture approximately 24 hours after Ca-DTPA

In Study 2, 22 of 76 cytokines changed significantly between the pre-chelation and approximately 24-hour samples in GDD.[9] Twenty decreased: IL-4, IL-16, MCP-3/CCL7, sICAM-1, eotaxin/CCL11, resistin, TPO, FGF-2, GRO-alpha/CXCL1, leptin, TSLP, ENA-78/CXCL5, IL-33, RANTES/CCL5, TGF-alpha, TNF-beta/LTA, CTACK/CCL27, IL-20, IL-22, and IL-1RA. Two increased: sFas/TNFRSF6 and G-CSF. The decreasing group was notably enriched for chemotaxis and cell-trafficking mediators, including GRO-alpha/CXCL1, ENA-78/CXCL5, MCP-3/CCL7, eotaxin/CCL11, RANTES/CCL5, CTACK/CCL27, IL-16, and the adhesion molecule sICAM-1. The broader pattern also involved inflammatory, epithelial/barrier, regulatory, hematopoietic, metabolic, and growth/repair signaling.

The small GSC comparison group did not demonstrate an analogous decrease cluster. None of the 22 cytokines that changed significantly in GDD showed a statistically significant decrease in GSC, and CTACK/CCL27 increased significantly in GSC.[9] This contrast is biologically notable but preliminary because only 3 unique GSC subjects contributed 4 paired treatment visits.

The raw-data descriptive reanalysis across 27 paired GDD treatment visits reproduced the direction of all 22 source-study changes. Median within-visit change was negative for each of the 20 cytokines originally reported to decrease and positive for both cytokines originally reported to increase. The largest median decreases were GRO-alpha/CXCL1 (-26.6%), ENA-78/CXCL5 (-23.4%), IL-4 (-11.7%), eotaxin/CCL11 (-10.2%), resistin (-8.9%), FGF-2 (-8.1%), TNF-beta/LTA (-7.4%), and MCP-3/CCL7 (-6.6%). Median increases were +11.3% for sFas/TNFRSF6 and +3.9% for G-CSF. Directional consistency was strongest for IL-4, IL-16, and sICAM-1, each decreasing in 22 of 27 visits; MCP-3/CCL7 and TNF-beta/LTA decreased in 20 of 27; and sFas/TNFRSF6 and G-CSF increased in 20 of 27. Detailed descriptive results are shown in Table 2 & Figure 2.

Table 2 Cytokines significantly changed from pre-chelation to approximately 24 hours in GDD and descriptive raw-data reanalysis of 27 paired GDD treatment visits

Cytokine	Predominant relevant function	Original P value	Original direction	Median raw change, %	Visits in original direction
IL-4	Adaptive/Th2-regulatory	0.003	Decrease	-11.7	22/27
IL-16	T-cell chemotaxis/trafficking	0.003	Decrease	-5.2	22/27
MCP-3/CCL7	Chemotaxis/monocyte recruitment	0.005	Decrease	-6.6	20/27
sFas/TNFRSF6	Apoptosis/immune regulation	0.004	Increase	+11.3	20/27
sICAM-1	Endothelial/adhesion	0.0004	Decrease	-2.4	22/27
Eotaxin/CCL11	Chemotaxis/eosinophil recruitment	0.01	Decrease	-10.2	16/27
Resistin	Inflammatory/metabolic	0.008	Decrease	-8.9	19/27
TPO	Hematopoietic/homeostatic	0.005	Decrease	-5.5	19/27
FGF-2	Growth/repair	0.02	Decrease	-8.1	17/27
GRO-alpha/CXCL1	Innate chemotaxis/neutrophil recruitment	0.02	Decrease	-26.6	18/27
Leptin	Metabolic/inflammatory	0.02	Decrease	-0.5	14/27
TSLP	Epithelial/Th2 signaling	0.02	Decrease	-4.1	18/27
ENA-78/CXCL5	Innate chemotaxis/neutrophil recruitment	0.03	Decrease	-23.4	19/27
IL-33	Alarmin/type 2 inflammation	0.03	Decrease	-3.2	19/27
RANTES/CCL5	Chemotaxis/T-cell recruitment	0.03	Decrease	-6.0	17/27
TGF-alpha	Growth/repair	0.03	Decrease	-1.5	15/27
TNF-beta/LTA	Inflammatory/lymphocyte signaling	0.03	Decrease	-7.4	20/27
CTACK/CCL27	Skin-homing chemotaxis	0.04	Decrease	-5.5	16/27
IL-20	Epithelial/inflammatory	0.04	Decrease	-4.7	16/27
IL-22	Barrier/epithelial/T-cell signaling	0.04	Decrease	-3.5	17/27
G-CSF	Myeloid/hematopoietic	0.04	Increase	+3.9	20/27
IL-1RA	Regulatory/anti-inflammatory	0.05	Decrease	-2.4	15/27

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Original P values and direction are from the Study 2 linear mixed-effects analysis.[9] Median raw change and directional counts are newly calculated descriptive statistics from the reconstructed 27 paired GDD treatment visits. No new inferential significance is assigned to these descriptive metrics. Functional categories are overlapping and context dependent.

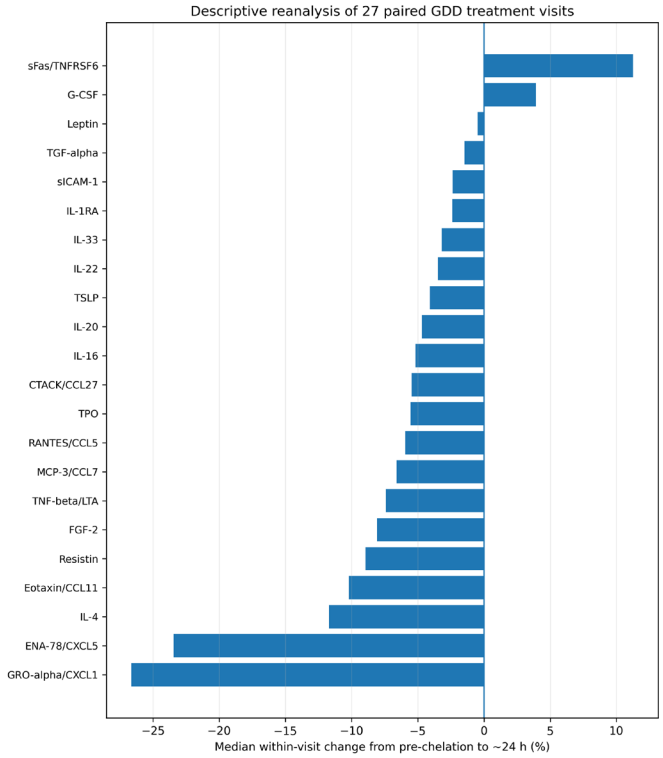


Figure 2 Descriptive raw-data reanalysis of the 22 cytokines previously identified as significantly changed from pre-chelation to approximately 24 hours in GDD. Bars show the median within-visit percent change across 27 paired GDD treatment visits. The direction of all 22 medians reproduced the direction of the original mixed-effects findings: 20 negative and 2 positive. These descriptive values are not new tests of statistical significance.

Dynamic and staggered cytokine responses

The dynamic study demonstrated that cytokine responses after Ca-DTPA were not synchronous.[10] EGF and sCD40L showed 30-minute peaks, as prospectively defined in that study, in at least 4 of 6 participants. ENA-78/CXCL5, eotaxin/CCL11, and leptin showed later 24-hour peaks in at least 4 of 6 participants. Three of the four GDD subjects contributed to the peak pattern for each of these early and late groups. The findings establish that a single post-chelation time point cannot represent the full cytokine response and support a staggered temporal sequence involving different signaling systems.

The relationship between the dynamic 24-hour peaks and the larger 24-hour pre/post cohort is informative. ENA-78/CXCL5, eotaxin/CCL11, and leptin were identified as 24-hour peaks in the dynamic study because they rose relative to the 60-minute value, whereas all three decreased from pre-chelation to approximately 24 hours in the larger Study 2 cohort. These observations are not mutually exclusive: a cytokine can fall during an early phase and subsequently rebound by 24 hours while remaining below its pre-chelation level, and the two studies also involved different subjects and exposure histories. The combined data therefore favor non-monotonic, temporally organized trajectories rather than a simple one-direction cytokine response.

Broad cytokine responders, gadolinium mobilization, and symptoms

In the six-subject dynamic study, 44 of 76 measured cytokines had at least one high-outlier participant, but the broad outlier pattern was concentrated largely in two individuals: one GDD subject and one asymptomatic GSC subject.[10] These two participants also had the highest post-chelation 24-hour urine gadolinium excretion (55 and 33 micrograms/24 h, respectively) and the greatest prior GBCA exposure in that cohort. The GDD broad responder had received MultiHance three times, with the most recent exposure approximately 4 months before chelation; the GSC broad responder had multiple prior GBCA administrations and no GDD symptoms despite a large cytokine response.[10]

A large systemic cytokine response that does not generate symptoms can therefore occur in an asymptomatic GSC subject. Conversely, all GDD subjects in the dynamic cohort experienced Flare, whereas neither GSC subject did.[10] These observations separate the magnitude of circulating cytokine signaling from the symptomatic phenotype and provide the basis for examining response composition, localization, and downstream organization as potentially more important determinants of disease expression.

Discussion

The three cytokine studies, when examined together rather than as isolated experiments, provide a coherent temporal view of GDD immune biology. GDD is associated with an altered baseline circulating cytokine state; Ca-DTPA-associated gadolinium mobilization perturbs that state; different cytokines become prominent at different intervals; and by approximately 24 hours a broad group of circulating cytokines is reduced in GDD, with no analogous statistically significant decrease cluster demonstrated in the small GSC comparison group. The central finding is therefore not simply that individual cytokines are high or low. It is that the cytokine response has architecture - a functional composition, a temporal sequence, and potentially an anatomical distribution.

The resting-state findings are an essential starting point. Study 1 identified a cluster of mostly elevated cytokines with a strong inflammatory and chemotactic component in GDD before any acute chelation challenge.[8] The second cohort also showed significant pre-chelation differences from healthy controls, but the specific analytes differed substantially and included both increases and decreases.[9] Only GRO-alpha was a shared elevated analyte. This should not be viewed as evidence against biological consistency. Cytokines are highly context dependent, and the two cohorts differed in exposure histories, time since disease onset, control populations, and assay panels. The more reproducible observation is an altered resting immune state, not a fixed diagnostic fingerprint composed of the same cytokines in every patient and every cohort.

The independent mechanistic literature provides a plausible explanation for the breadth of the resting cytokine findings. In human-derived macrophage systems, GBCA exposure has been associated with NF-κB-mediated chemokine production and TLR-dependent proinflammatory signaling.[11,12] In murine systems, gadolinium-containing compounds can engage the NLRP3 inflammasome and promote IL-1β release and inflammatory-cell recruitment.[13] Lower-concentration in vitro experiments also indicate that the direction and magnitude of oxidative and cytokine responses depend on the

specific agent and exposure conditions.[14] These data do not identify a single obligatory pathway in GDD; rather, they support interpretation of the observed clinical cytokine changes as potentially arising from interacting innate immune programs.

The approximately 24-hour state is particularly informative. Twenty of 22 significant changes in GDD were decreases, and the new raw-data descriptive reanalysis reproduced the direction of every source-study change across the 27 GDD treatment visits. The decrease cluster was enriched for chemokines and trafficking/adhesion mediators. This functional enrichment strengthens the mechanistic interpretation proposed in the original Study 2 report: lower serum cytokine concentrations during Flare need not signify less inflammation.[9] Instead, the pattern may reflect, at least in part, redistribution of activated cells and signaling from the circulation toward tissue compartments where retained or newly mobilized gadolinium is being encountered.

This interpretation is analogous, but not identical, to observations in other inflammatory diseases. In rheumatoid arthritis, circulating pre-inflammatory mesenchymal (PRIME) cells decrease during clinical flares and have been proposed to traffic toward inflamed synovial tissue.[20] The GDD data do not demonstrate PRIME-cell migration, and no tissue cytokine or cell-trafficking measurements were obtained. The relevance of the rheumatoid arthritis model is conceptual: a fall in a circulating inflammatory signal can occur while tissue-level disease activity is increasing. The GDD hypothesis should therefore be framed as redistribution or compartmentalization of immune activity rather than as proven migration of a specific cell population.

Macrophage experiments offer an additional, but indirect, point of comparison. Following experimental exposure to gadoterate, investigators documented persistence of intracellular gadolinium together with alterations in RhoA/mTOR signaling and organelle markers; later work found changes in adhesion, motility, and chemokine-associated expression.[16,17] Studies incorporating a magnetic-field gradient also reported altered macrophage morphology and inflammatory polarization, although the magnetic-field and relatively high-concentration culture conditions limit extrapolation.[18] Gadolinium-associated calcium-phosphate precipitation has independently been associated with profibrotic macrophage activation *in vitro*.[15] None of these studies measured trafficking of immune cells in GDD patients. They support the plausibility of tissue-compartment effects without establishing that the 24-hour serum decreases in the present datasets resulted from cellular migration.

The dynamic study adds a crucial temporal dimension to this model.[10] Cytokine release following Ca-DTPA is not a single synchronized burst. EGF and sCD40L became prominent at approximately 30 minutes, whereas ENA-78/CXCL5, eotaxin/CCL11, and leptin showed later 24-hour peaks. This means that the 24-hour blood draw in Study 2 represents a later phase of an evolving response, not the first biological event after chelation. A cytokine measured as lower than baseline at 24 hours may have passed through an earlier rise, fall, or partial rebound that a two-time-point design cannot detect. The apparent divergence between the 24-hour peaks of ENA-78, eotaxin, and leptin in the dynamic study and their pre-to-24-hour decreases in the larger cohort is therefore informative rather than contradictory: the reference time points differ, and the trajectories can be non-monotonic.

The GSC observations further refine the model. A broad systemic cytokine response occurred in an asymptomatic GSC subject, demonstrating that the magnitude of circulating cytokine activation alone is insufficient to generate the GDD phenotype.[10] At the same time, the larger pre/post study found a coordinated 24-hour decrease pattern in GDD that was not statistically reproduced in GSC.[9] Because the GSC sample is very small, this cannot yet be regarded as a definitive disease discriminator. It is nevertheless a compelling preliminary observation: symptomatic GDD may depend more strongly on which pathways are activated, when they are activated, where responding cells and mediators are localized, and what downstream tissue programs they initiate than on the absolute height of a serum cytokine peak.

The broad-responder data also suggest that the amount and accessibility of retained gadolinium may influence systemic cytokine magnitude. In the dynamic cohort, the two broad cytokine high-outliers were the two highest post-chelation gadolinium excreters and had the greatest prior GBCA exposure.[10] One was symptomatic GDD and the other asymptomatic GSC. The GDD broad responder had multiple relatively recent GBCA exposures, whereas the GSC broad responder responded years after the last GBCA. This pattern is consistent with a long-lived retained gadolinium reservoir that can remain biologically perturbable by DTPA. Cytokine measurements alone cannot establish the anatomical source or chemical speciation of mobilized gadolinium, but the temporal response after chelation is more consistent with perturbation of retained tissue or other extravascular reservoirs than with a large continuously circulating gadolinium pool.

Evidence from other preclinical settings further supports the distinction between circulating markers and compartment-specific tissue responses. In contrast-agent exposure models involving cultured fibroblasts and renally impaired rodents, investigators reported tissue-dependent fibrotic changes despite widespread gadolinium detection.[21] Other experimental NSF-associated models implicated NOX4-linked oxidative stress and neutrophil elastase in inflammatory or fibrotic tissue responses.[22,23] These investigations concern different disease settings, particularly impaired renal function and NSF-like pathology, and must not be treated as demonstrations of the mechanisms responsible for GDD in patients with normal renal function.

Experimental work in additional organs broadens the biological context but also illustrates the limits of inference. Repeated-GBCA studies in rats have reported neuroinflammatory and oxidative-stress changes, including NLRP3-associated signaling under diabetic conditions; other mouse experiments have described agent-specific multiorgan changes.[24–26] In vitro work with human proximal-tubule cells exposed to gadolinium ions has identified oxidative, mitochondrial, and inflammation-related signaling responses, and an independent review summarizes the diversity of reported toxicological mechanisms.[27,28] These findings cannot establish a causal relationship between any particular circulating cytokine profile and clinical GDD. Their relevance here is that gadolinium-associated biological effects may differ across cell types, organs, chemical species, and time points.

This integrated interpretation also situates GDD within a general heavy-metal toxicity framework.[5] Gadolinium is not metabolized into a harmless element; it must remain coordinated, become re-coordinated, be sequestered, or be eliminated. DTPA creates a defined perturbation by markedly increasing urinary gadolinium elimination in both GDD and GSC.[9,10,29] The cytokine studies therefore have an unusual experimental advantage for a chronic toxicologic illness: the suspected retained toxicant can be acutely mobilized, and biological responses can be observed against a measurable decorporation event. This does not by itself establish that every cytokine change is caused

directly by gadolinium, but it provides a mechanistically informative challenge-response model.

Other human biomarker work has reported altered functional mitochondrial proteins in plasma neuron-derived extracellular vesicles from individuals with gadolinium deposition.[30] That observation concerns a different biological compartment and assay platform; it is complementary context rather than confirmation of a causal cytokine-to-symptom mechanism.

Several limitations are important. The three studies were not originally designed as one unified experiment. They used different cytokine panels, control cohorts, batches, and sampling schedules, so absolute MFI values cannot be pooled across studies without qualification. The baseline studies lacked pre-GBCA cytokine measurements, preventing determination of whether resting abnormalities represent pre-existing susceptibility, consequences of retained gadolinium, or both. Study 2 was exploratory and did not correct its multiple pre/post comparisons for multiplicity. The GSC comparison groups were very small, especially for inferential analysis. Study 3 included only six subjects and was designed to identify kinetic patterns rather than establish disease-specific trajectories. Serum cytokines are also imperfect surrogates for tissue immune activity. Finally, functional cytokine categories overlap and should be viewed as an interpretive framework rather than fixed biological labels.

Despite these limitations, the integration of the three datasets yields a testable model. GDD appears to have both a state component and a response component: an altered resting cytokine milieu and an organized response to acute gadolinium mobilization. Future prospective studies should enroll larger, exposure-matched GDD and GSC cohorts; collect baseline, minute-scale, hour-scale, and 24-hour specimens; measure circulating immune-cell subsets alongside cytokines; and, where feasible, obtain tissue-level or other compartment-specific evidence. Such designs could directly test whether the 24-hour circulating decrease corresponds to tissue recruitment and whether functional module trajectories distinguish symptomatic from asymptomatic gadolinium retention.

Conclusion

Integrated analysis of three human cytokine studies supports a model of GDD as a disorder with an altered resting cytokine architecture and a temporally organized response to Ca-DTPA-associated gadolinium mobilization. The response is staggered rather than synchronous. By approximately 24 hours, a broad cluster of circulating cytokines - particularly chemotactic and trafficking-related mediators - decreases in GDD, whereas an analogous statistically significant decrease cluster was not observed in the small GSC group. A large systemic cytokine response can occur without symptoms in GSC, indicating that cytokine magnitude alone does not define clinical disease. The emerging distinction between GDD and asymptomatic gadolinium retention may therefore reside in cytokine composition, timing, tissue localization, and downstream organization. Larger prospective GDD-GSC studies are warranted to test this model directly.

Data availability

The present work uses data generated in the three cited source studies. Deidentified source data and analytical records are maintained by the investigators; sharing is subject to applicable institutional, ethical, and data-use requirements.

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Conflicts of interest

Author declared no conflicts of interest.

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