Cytokine storm complicated by cardiogenic shock induced by anti-HER2 therapies

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ABSTRACT
Cytokine storm induced by anti-human epidermal growth factor receptor-2 (HER2) therapies has not been reported. We report a patient with breast cancer treated with trastuzumab/pertuzumab who developed severe biventricular dysfunction and cardiogenic shock (CS) 6 months after starting double anti-HER2 therapy. The CS was accompanied by severe systemic inflammation, and cardiac MRI (cMRI) showed structural changes typical of myocardial inflammation. The immuno-inflammatory profile showed significantly increased levels of activation of the complement system, proinflammatory cytokines (IL-1β, IL-6, IL-18, IL-17A, TNF-alpha) with increased activity of classical mononuclear, T helper 17 cells (Th17), CD4 T and effector memory CD8 T subsets, whereas NK cell activation was not observed. The data suggest an important role for monocytes as initiators of this FcγR-dependent antibody-dependent cytotoxicity, leading to the overactivation of an adaptive T cell response, in which Th17 cells may act in synergy with Th1 helper cells to drive the severe cytokine release syndrome. After discontinuation of trastuzumab/pertuzumab, hypercytokinemia and complement activity normalized along with clinical recovery. Cardiac function returned to baseline within 2 months of initial presentation, together with a resolution of the myocardial inflammation on MRI.

BACKGROUND
About 15%–20% of patients with breast cancer (BC) overexpress the HER21 and modulation of tumor immunity by therapeutic monoclonal antibodies has been a success story in HER2-positive BC treatment.2 Antitumor mAbs have the capacity to elicit several mechanisms of antitumor action such as direct antitumor activity (blockade of a specific signaling molecule to inhibit oncogenic cellular signaling, or indirect antitumor activity through the engagement with FcγR on immune cells to induce Antibody-dependent cell-mediated cytotoxicity (ADCC)).1,2 In addition, antibody-dependent phagocytosis or activation of serum proteins, such as the complement family, allow stimulation of different immune cells such as macrophages, neutrophils and NK cells which can further activate adaptive immune responses through antigen presentation, cytokine production and chemotaxis.

In HER2-positive BC, the combination strategy with two HER2 specific monoclonal antibodies (mAbs), trastuzumab and pertuzumab3 was shown to be superior to trastuzumab treatment alone with respect both to tumor progression and overall survival in the metastatic setting3–5 and rate of pathological complete response in the neoadjuvant setting.6 The synergistic mechanism-of-action (MOA) remains incompletely understood, however, increase of ADCC has been reported.7–9 However, since trastuzumab also activates NK cells10 resulting not only in discharge of granzymes and perforins but also in release of the proinflammatory cytokines such as IFN-γ and TNF-α (ADCR),2,11 a complementary action may contribute to the success of double therapy in HER2-positive BC.

INSIGHT
Here, we describe the first case of cardiogenic shock (CS) following a double anti-human epidermal growth factor receptor-2 (HER2) blockade-induced cytokine storm involving monocytes as initiators of an FcγR receptors (FcγR)-dependent antibody-dependent cytotoxicity (ADCR) with a pathogenic role for the activation of the inflammasome and the Th17/Th1 axis. When compared with the immune checkpoint inhibitors (ICI)-induced CS, important differences were found, including an increase in IL-10 and the absence of an increase in IFN-γ or NK cell activation, suggesting an important difference in the regulation of the immune response in terms of proinflammatory and counter-regulatory anti-inflammatory CARs (compensatory anti-inflammatory response).
However, no inflammasome or hypercytokinemia-related cardiovascular toxicity has been reported with double antiHER2 therapy in HER2-positive BC, although trastuzumab has been associated with significant drug-induced ventricular dysfunction (0.6%–4.5%), and this incidence is even higher (34%) when patients with BC have previously received anthracycline-based chemotherapy.12

**CASE REPORT**

This patient, diagnosed with metastatic endocrine receptor positive BC, was started on trastuzumab-pertuzumab therapy associated with weekly paclitaxel when liver metastasis histology revealed positive expression of HER2. Chemotherapy was discontinued after six cycles and trastuzumab-pertuzumab therapy was associated with endocrine therapy. At the beginning of double antiHER2 therapy, the patient had a mildly decreased left ventricular ejection fraction (LVEF) (48%) related to prior doxorubicin chemotherapy (cumulative dose of 300 mg/m2) and adolescent mediastinal radiotherapy for nodular sclerosis classic Hodgkin's lymphoma. Tropolin and NT-proBNP levels remained normal during follow-up.

Seven months later and 3 weeks after the last cycle of trastuzumab-pertuzumab, the patient was admitted with CS, severe dyspnea, prominent right-sided fluid retention, and biological signs of renal and hepatic dysfunction (L-lactate: 10 mmol/L (N=0.63–2.44 mmol/L), creatinine: 1.7 mg/dL (N=0.9 mg/dL), ASAT: 11,657 U/L (N=32 U/L), ALAT: 2,653 U/L (N<36 U/L), total bilirubin: 1.8 mg/dL (N=0.12 mg/dL), factor V: 7% (N 70%–180%). Blood analysis marked the presence of severe systemic inflammation (CRP: 101 mg/L (N<10 mg/L), ferritin: 49,291 µg/L (N 15–150 µg/L), d-dimers: 34,432 ng/mL (N<500 ng/mL) suggesting cytokine release syndrome (CRS). Complement activation were identified with consumption of C3c: 0.52 g/L (N=0.75–1.4 g/L), C4: 0.06 g/L (N=0.10–0.34 g/L), and elevation of SC5B9: 1078 ng/mL (N=127–303 ng/mL) and b2b factor: 2.92 µg/mL (N<1.65 µg/mL). A large infected panel was negative including; COVID-19, CMV, VZV, EBV, HSV 1 and 2, hepatitis A/B/C/E, HIV, blood and urine cultures. In addition, there was no evidence of sepsis and no empirical antibiotic therapy was given. Cardiac MRI (cMRI) noted mild pericardial effusion, the LV (left ventricular) was dilated, LVEF was severely reduced to 28%, right ventricular systolic function was 36%. T2-mapping revealed values >60 ms of the LV posterior wall suggesting myocardial edema compatible with late gadolinium enhancement in the subepicardium of the same region (figure 1).

In order to select treatment options for the best management of CRS and CS, the immune-inflammatory profile of our patient was determined. Serum levels of a large panel (n=48) of mediators, including cytokines, soluble cytokine receptors, chemokines and growth factors, were determined from blood samples collected at admission (visit 1=V1) and the same panel was applied for follow-up (visits 2–5=V2-5). The normal range of values for each of these 48 markers was defined on the basis of reference results obtained in sera from 450 healthy individuals serving as controls.12 An age-matched BC control patient, also treated with antiHER2 trastuzumab/pertuzumab, served as control. Serum levels of twelve cytokines and soluble cytokine receptors (IL-1RA, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-10, IL-15, IL-17A, IL-18, and TNF-α), four chemokines (CCL2, CCL5, CXCL8, CXCL9, and CXCL13) and seven growth factors (EGF, HGF, LIF, PDGF-BB, VEGF-D, GM-CSF, and G-CSF) were significantly upregulated at visit 1 compared with the other time points and compared with the healthy controls and the BC control (figure 2A). In addition, the cytokines and growth factors IL-1RA, IL-6, IL-15, IL-17A, IL-18, EGF, HGF, LIF, PLGF, SCF, GM-CSF, and G-CSF were still detectable in the serum of the patient at visit 2 and visit 3 and decreased at visit 4 and visit 5.

We first analyzed the absolute blood counts of CD4, CD8 T cells, B cells, NK cells and monocytes to better understand the immune cell populations involved in the massive cytokine release in our patient. Interestingly, we observed between visit 1 and 2 strong lymphocytosis of CD8 T cells (CD8: V1=525 cells/mm3; V2=1678 cells/mm3) and B cells (CD8 cells V1=483 cells/mm3; V2=638 cells/mm3) (figure 2B) and elevation of monocyte counts beyond the standard reference interval for healthy donors at both visits (monocytes: V1=1180 cells/mm3; V2=1988 cells/mm3). In addition, the blood CD4 T-cell count increased 1.6-fold but was still within the normal reference interval (figure 2B). Otherwise, our patient showed a significant decrease in blood NK counts at visit 1 (NK: V1=62 cells/mm3; V2=103 cells/mm3).

These changes were different to the BC control presenting normal values for monocytes, NK and CD8 T cells (NK=131 cells/mm3, CD8 T=338 cells/mm3, monocytes=341 cells/mm3) while CD4 T cells and B cells were below the value of the standard reference interval (CD4 T=321 cells/mm3, B cells=21 cells/mm3).

Having identified alterations in the absolute immune cell counts in blood, we next performed mass cytometry to investigate the distribution of CD4 and CD8 T cell subsets, NK cells and monocyte populations in the case and the BC control patient and compared their profile to age-matched healthy controls (n=20). Cumulative data indicated that our patient showed 3.6-fold and 4.6-fold proportional increase of Th1 CD4 T cells at visit 1 and visit 2, respectively, when compared with healthy controls (CTRL); the increase was twofold when matched with the BC control (V1: 50%; V2=56.7% vs CTRL=12.2%; BC=24%). In contrast, frequencies of Th2 cells (V1: 20.4%; V2=8.8% vs CTRL=30.6%; BC=24%) were decreased at visit 2 in our patient relative to controls; in accordance, T regulatory cells were slightly decreased at visit 1 as compared with controls (V1: 2.7%; V2=5.5% vs CTRL=5.5%; BC=6%) (figure 2C). Similar levels in the proportion of Th17 (V1: 9.8%; V2=10.4% vs CTRL=2.3%; BC=14%) and Th1/Th17 (V1: 7.6%; V2=15.8% vs...
CTRL=1.3%, BC=12%) CD4 T cell populations were found between the patient and the BC control but upregulated as compared with the healthy controls.

We then assessed the distribution of CD8 T cells subsets based on CCR7, CD27, CD45RA expression to identify naïve (CCR7+, CD27+, CD45RA+), central memory (CM: CCR7+, CD27+, CD45RA−), transitional memory (TM: CCR7−, CD27+, CD45RA−), effector memory (EM: CCR7−, CD27−, CD45RA−) and EM cells re-expressing CD45RA (EMRA: CCR7−, CD27−, CD45RA+). Cumulative data showed that the patient had increased proportion of EM CD8 T cells at visit 1 when compared with healthy controls (V1=58%, V2=37.3%, CTRLS=25.3%; BC=13%); while EMRA CD8 T cells were upregulated in the patient at visit 1 and visit 2 relative to healthy controls but not with respect to the BC control (V1=30%, V2=48%, CTRLS=14.3%; BC=61%). The patient showed a concomitant decrease in the frequencies of the CM and TM CD8 T cell populations (CM: V1=5%, V2=2.8%, CTRLS=24.2%, BC=3%; TM: V1=5.3%, V2=10.9%; CTRLS=50.6%, BC=15%) (figure 2D).

Next, we measured the proportion and profile of NK cells. The proportion of CD56+CD16+ and CD56brightCD16− NK cells was not different in the case patient compared with controls (CD56+CD16+: V1=89%, V2=90%, CTRLS=90%, BC=90%; CD56brightCD16−: V1=10%, V2=5%; CTRLS=8.6%, BC=10%) (figure 2F) and no major differences were found in the expression of several activation markers (CD8, CD11c, CD27, CD38, CD62L, CD25) at V1 and V2 in the case patient when compared with controls (figure 2F).

Finally, we measured the monocyte subsets, which are divided into three major populations: classical (CL: CD14+CD16−), non-classical (NCL: CD14dimCD16+) and intermediate (ITM: CD14+CD16+). The case patient had an increased proportion of classical monocytes (CL: V1=83.4%, V2=90%; CTRLS=69%, BC=78%) and very low to undetectable frequencies of ITM and NCL monocytes (ITM: V1=3%, V2=4%; CTRLS=18%, BC=10%; NCL: V1=3.3%, V2=0.8%; CTRLS=12.2%, BC=11%) compared with controls (figure 2G). Furthermore, the expressions of several membrane receptors associated with monocyte functional activity and cell homing were found to be altered on classical monocytes at visit 1 and partially restored at visit 2 (CD31, CD1c, CXCR3 and CD11c), whereas the levels of HLA-DR and CD38 remained low at visit 2 (HLA-DR mean intensities: V1=1.3, V2=2.6, CTRLS=25.6, BC=26; CD38 mean: V1=15, V2=26, CTRLS=40, BC=41) (figure 2H).
On this basis, CS triggered by anti-HER2 therapy-induced CRS was diagnosed. The patient stabilized within 5 days with temporary inotropic and vasoconstrictor support combined with intravenous diuretics, in parallel with gradual resolution of inflammatory markers and cytokines levels (figure 1). Thus, immunosuppressive treatment with systemic corticosteroids was not applied since CRS due to trastuzumab-pertuzumab treatment has not yet been described in the literature and in our case, the implication of cardiodepressant cytokines was demonstrated only after recovery from CS. The clinical improvement was further assisted by the reestablishment of guideline-directed HF therapy resulting in an increase of the LVEF to 42% and, importantly, normalization of RVEF conjoint with disappearance of myocardial edema.

**DISCUSSION**

Though the role of reactive oxygen species is well acknowledged, it remains unknown whether hypercytokinemia syndrome and complement activation are of clinical importance in cardiotoxicity resulting from anti-HER2
therapy. Members of the IL-1 family such as IL-1β and IL-18 are known to promote LV dysfunction in septic cardiomyopathy. However, a cytokine storm secondary to anti-HER2 blockade with activation of inflammasome and complement has not been described so far.

When bound to their antigen on the cell surface, anti-HER2 mAbs can recruit through their Fc region various FcγR-expressing immune cells present in the tumor microenvironment. These cells are macrophages, neutrophils and NK, and triggered FcγR-dependent MOA are: (1) ADCC, (2) phagocytosis of mAbs-opsonized target cells, and (3) cytokine and soluble inflammation mediators release. The production of various proinflammatory cytokines such as TNF-α, IFN-γ, IL-18, IL-1β and IL-6 following IgG-FcγRs interaction is likely to contribute to the mobilization and activation of a large variety of immune cells following anti-HER2 treatment triggering a ‘cytokine storm’. In the case patient we observed principally an activation of the classical monocytes, as demonstrated through reduced cell surface expression of HLA-DR, CD38 and CD31, whereas the NK cells were not activated, suggesting a principal role for monocytes as initiator of this FcγR-dependent ADCR, leading to the overactivation of T cells and Th1, Th17 and Th1/17 CD4 T cells and EM CD8 T subsets concomitant with complement activation and consumption.

The hypercytokinemia was mainly caused by proinflammatory Th1 and Th17 cytokines (IL-1β, IL-6, IL-18, IL-17A, TNF-alpha). Taken together, the data suggest an important role for monocytes as initiator of this FcγR-dependent ADCR, leading to overactivation of adaptive T cells, in which Th17 cells may act in synergy with Th1 cells to drive the severe CRS. Moreover, the patient did not meet the criteria for reactive hemophagocytic syndrome such as macrophage activation syndrome (MAS) or hemophagocytic lymphohistiocytosis (HLH). Indeed, the Hscore14 was 102 points, which makes the probability of an MAS or HLH very low (1%–3%). Interestingly, we observed an excessively high ferritinemia (>49,000, N<150 μg/L) with a strong hepatic cytolyis but without cytopenia, fever or organomegaly and a normal fibrinogen level. It is also important to note the presence of a spontaneously resolved systemic coagulation activation without fulfilling the criteria of disseminated intravascular coagulation. The patient had a normal fibrinogen level despite a very high dimer level (>34,000, N≤500 ng/mL) along with the consumption of coagulation factors II, V, VII and X.

Compared with the cytokine storm associated with other therapeutic antibodies, such as ICI,15-16 the anti-HER2 mAbs-related cytokine signature here was characterized by multiple variations, including concomitant increases in TNF-α, IL-1β and IL-18, as well as GM-CSF and G-CSF, while there was no increase in IFN-γ and CXCL12, and a smaller increase in IL-6 levels. IL-1β is an inducer of the expression of IL-17, IL-21 and IL-22. Here, we did not observe an increase in the expression of IL-21 and IL-22, indicating that IL-1β is rather involved in the modulation of cardiac inflammation as suggested by elevation of the proinflammatory cytokines IL-1β and IL-18 strongly likely due to activation of inflammasomes. Interestingly, despite the elevation of IL-1β and IL-18, we did not find IFN-γ secretion or NK cell activation. IL-10 is the cytokine mainly produced by T regulatory lymphocytes (Tregs) involved in the dampening of immune responses and the inhibition of the main proinflammatory cytokines. Another interesting pathogenic feature is that previous studies published by our group have shown that there is no increase in IL-10 in ICI-related CS.16 This may be related to the depletion of Tregs often observed in patients treated with ICI. We further observed a rapid normalization in 48–72 hours characterized by a strong decrease of ferritinemia to 8000 (vs 49,000) in parallel with a massive increase of anti-inflammatory cytokines IL1Ra>2700 and a very strong decrease of proinflammatory cytokines IL6 and IL-18 and immune activation. This difference may indicate a different regulation of the immune response during the natural CARS that occurs during anti-HER2 CS and is characterized by a simultaneous increase in both IL-1Ra and IL-10, indicating a more potent natural CARS that requires no administration of immunosuppressants, in contrast to the high dose of immunosuppressants required to treat ICI-associated CS.

The case patient had not only a new onset of severe biventricular dysfunction leading to CS, but also signs of severe systemic inflammation and cytokine storm in the blood complicated by myocardial inflammation in eMRI. IL-1β and IL-18 levels normalized with clinical recovery and in parallel with reestablishment of cardiac function back to baseline 2 months after the initial presentation with CS. Of note, similar changes have been reported in a mouse model testing the effect of these cardio depressive cytokines.13

Pathophysiological background for their increased concentration in the bloodstream and in the myocardium is chemotherapy-induced activation of the inflammasome, which processes precursors of IL-1β and IL-18 into their active forms.13 Therefore, IL-1 blockade may be a therapeutic option, and Anakinra, for instance, is a recombinant form of the naturally occurring IL-1Ra which has already been shown to improve outcome in acute myocardiitis refractory to standard treatment.13

In CRS, a particular concern is acute heart failure, which is usually of rapid onset and associated with severe cardiac dysfunction, most often transitory, and similar to what we describe in our case report. The pathophysiology of acute cardiac toxicity in CRS is unclear, but is similar to sepsis-related cardiomyopathy and stress cardiomyopathy, also known as Takotsubo cardiomyopathy.18 The timing of the onset of symptoms and the severity of CRS are dependent on the inducing agent and the degree of immune cell activation. In addition, it has been reported that the onset of symptoms can occur from days to occasionally weeks after the administration of treatment.18

The endomyocardial biopsy was considered by not undertaken as the clinical evolution was rapidly favorable. In theory, one may consider acute stress-induced (Takotsubo) cardiomyopathy as differential diagnosis. Takotsubo cardiomyopathy is accompanied by myocardial and systemic inflammatory activation. Myocardial uptake of USPIO (cardiac MRI with intravenous infusion of ultrasmall superparamagnetic particles of iron oxide) was higher in patients in the acute phase of Takotsubo than in control individuals, suggesting a macrophage-driven cellular infiltration in the myocardium. Patients with acute Takotsubo also present elevated levels in the blood of the proinflammatory cytokines IL-6, IL-8 and CXCL1, an increase in proinflammatory monocytes (CD14++CD16−) and a decrease in intermediate (CD14+CD16+) and nonclassical (CD14+CD16++) monocytes. USPIO enhancement was no longer detectable after 5 months of the index event, but some of the systemic changes persisted, such as increased IL-6 levels and decreased number of intermediate monocytes. Since we do not have evidence of ischemic or infectious cardiomyopathy in our patient, the CS and myocarditis may be secondary to Takotsubo cardiomyopathy. However, this diagnosis is not proposed on the basis of MRI-based imaging which misses changes characteristic for Takotsubo cardiomyopathy.

Otherwise, a possible part related to a concomitant drug-induced cardiotoxicity by trastuzumab cannot be fully ruled out. In clinical routine, trastuzumab-related cardiotoxicity has always remained a reason of concern prompting the exclusion of patients with LVEF<50%.

Drugs like paclitaxel could induce a strong immune response which can be further augmented by the administration of anti-cytokine immunosuppressive therapy by IL1-blockade.

**Contributors** M0, RG and AN wrote the manuscript and prepared the figures and tables. AN, CF and MO did the immuno-profiling analysis. RG, RH and SH did the heart analysis. AS and SP did the oncology treatment. All authors commented on and corrected the manuscript.

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