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Analysis of cytokine release assay data using machine learning approaches

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ABSTRACT

The possible onset of Cytokine Release Syndrome (CRS) is an important consideration in the development of monoclonal antibody (mAb) therapeutics. In this study, several machine learning approaches are used to analyze CRS data. The analyzed data come from a human blood in vitro assay which was used to assess the potential of mAb-based therapeutics to produce cytokine release similar to that induced by Anti-CD28 superagonistic (Anti-CD28 SA) mAbs. The data contain 7 mAbs and two negative controls, a total of 423 samples coming from 44 donors. Three (3) machine learning approaches were applied in combination to observations obtained from that assay, namely (i) Hierarchical Cluster Analysis (HCA); (ii) Principal Component Analysis (PCA) followed by K-means clustering; and (iii) Decision Tree Classification (DTC). All three approaches were able to identify the treatment that caused the most severe cytokine response. HCA was able to provide information about the expected number of clusters in the data. PCA coupled with K-means clustering allowed classification of treatments sample by sample, and visualizing clusters of treatments. DTC models showed the relative importance of various cytokines such as IFN-γ, TNF-α and IL-10 to CRS. The use of these approaches in tandem provides better selection of parameters for one method based on outcomes from another, and an overall improved analysis of the data through complementary approaches. Moreover, the DTC analysis showed in addition that IL-17 may be correlated with CRS reactions, although this correlation has not yet been corroborated in the literature.

1. Introduction

Cytokine Release Syndrome (CRS) is an adverse event resulting in systemic release of cytokines in humans upon exposure to therapeutic mAbs. Cytokine release reactions in humans range from mild to severe, with symptoms such as fatigue, headache, urticaria and asthenia among others [1]. Some CRS reactions can be controlled by slowing the infusion rate of the mAb or by administering anti-inflammatory drugs [2]. However, when applied to human volunteers in a 2006 phase I clinical trial, the Anti-CD28 SA mAb TGN 1412 caused unexpected serious adverse events; healthy volunteers developed severe CRS within 90 min of receiving a dose of Anti-CD28 SA [3]. These adverse events were not predicted by pre-clinical safety assessments using animal testing of the drugs [4].

Both non-human primates and rodents are relatively resistant to CRS. Although release of cytokines has been observed in animal models, rarely did it progress to clinically relevant levels [5–8]. Differences in expression of target molecules, regulatory T cells, cytokines required for inflammatory response, and cell surface receptors among humans, rodents and non-human primates [9–14] all indicate that it may not be appropriate to use animal models to predict CRS in humans. To further understand CRS in humans, an in vitro assay using human whole blood was developed and tested by Walker et al. [2]. This assay was designed to support First-In-Human readiness, assessing the potential for mAbs to release of cytokines similar to Anti-CD28 SA. The study reported in this paper has used this assay for further analysis of CRS using several machine learning approaches.

The assay tests mAbs that have been immobilized on Protein-A-coated polystyrene beads and incubated with whole human blood cultures to stimulate cytokine release. Following incubation, supernatants were harvested and frozen for later multiplex analysis of cytokines using Searchlight™ technology. The assay was used by Walker et al. to compare the release of 11 cytokines[1] from Anti-CD28 SA (clone

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[1] 12 cytokines were measured in the assay. However, Walker et al. [2] showed no statistically relevant difference in the concentration of IL-8 release produced by AutoPlasma and Anti-CD28 SA. Therefore, IL-8 was not considered further, leaving 11 cytokine levels to be analyzed.

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ANC28.1/5D10) to those released by 8 other mAbs and controls using Hierarchical Cluster Analysis (HCA) [2]. In the present study, we analyze a data set with 7 mAbs and 2 negative controls. They consist of (1) PBS (no beads), which should not induce cytokines; and (2) beads coated with autologous plasma (AutoPlasma) from the same donors. The 7 mAbs, which are listed in Table 2, included 5 marketed mAbs whose ability to elicit CRS is known, and 2 research grade mAbs whose ability to elicit CRS is not known. Based on the mechanism of action of the mAb, we have assigned these two "unknown" mAbs to the "Safe" category.

The objectives of our study, using this in vitro assay, were: 1) to determine whether the cytokine response from a given mAb is similar to the severe cytokine response to Anti-CD28 SA; 2) to compare the utility of different machine learning approaches on the data collected from the assay; and 3) to determine the cytokines that are highly relevant for CRS detection. To achieve these objectives, three machine learning approaches were applied to the data set. They are: 1) Hierarchical Cluster Analysis (HCA) [15]; 2) Principal Component Analysis (PCA) [16] followed by K-means clustering [16]; and 3) Decision Tree Classification (DTC) [17,18].

Hierarchical Cluster Analysis (HCA) is a statistical method which builds a hierarchy of clusters based on the similarity between samples in a data set. This hierarchy is often displayed by means of a dendrogram. HCA has been widely used to analyze various cytokine responses, for example, it was applied to group the pathogen exposures based on the multivariate cytokine expression profiles induced in a host infection model system [19].

Principal Component Analysis (PCA) is a method that identifies patterns in high dimensional data and expresses these data in a way that highlights their similarities and differences. The procedure uses an orthogonal transformation to convert the observations, which are presented as functions of possibly correlated variables, into a set of values of linearly uncorrelated variables called Principal Components [20]. PCA has been used to analyze cytokine responses in multiple studies [21–23]. K-means clustering partitions a set of observations (which in our case emanate from PCA) into a set of clusters. Each observation is placed in the cluster with the nearest mean (the mean of the cluster serves as its prototype). K-means clustering has also been used before for cytokine data analysis [24–26].

Decision Tree Classification (DTC) is a nonparametric statistical supervised learning method that incorporates feature selection and possesses intuitive interpretability [27]. It maps observations about an item into conclusions about the item's target value. DTC has been used in several studies to analyze data related to cytokine response. McKinney et al. [28] used DTC to study the serum cytokines that have the strongest association with systemic adverse event of vaccinia virus. Patel et al. [29] used DTC to obtain a model that classifies subjects based on 17 cytokines into a respiratory syncytial virus (RSV)-induced acute otitis media (AOM) group and an early treatment failure group, respectively.

Each of the machine learning techniques used in our study was employed in some form in past investigations of cytokine response. What is demonstrated here, however, is the advantage of using these techniques in tandem. This advantage includes better selection of parameters for one method based on outcomes from another, and an overall improved analysis of the data through complementary approaches.

2. Materials and methods

2.1. Experimental design

We used data from the in vitro assay described by Walker et al. [2] to study the cytokine concentrations elicited by various mAbs. For this assay, blood was drawn aseptically under informed consent[2] by venipuncture using a 21-gauge needle from 44 normal human volunteers into BD Heparin Vacutainer (San Jose, CA) tubes. Cultures were set up within 2 h of blood collection. Previous reports on these types of assays suggest the need to immobilize Anti-CD28 for maximal cytokine production [4]. For this purpose, Protein A coated polystyrene beads were selected. Beads were coated with a saturating amount of mAb and then distributed to a 96-well culture dish. Each well contained 1×107 beads/well along with 200 μl of 1:10 diluted whole blood in RPMI 1640 media. After 48 h of culture, plates were centrifuged and supernatants were collected, frozen, and stored for future multiplex cytokine analysis.

We used the assay to test the stimulation of human blood from different donors where the application of a given treatment (mAb) on blood from a particular donor constituted a sample. The concentrations of the 11 cytokines shown in Table 1 were measured for each sample. These concentrations were measured in triplicate by multiplex enzyme-linked immunosorbent assay (ELISA) using SearchLight™ technology from Aushon Biosystems (Billerica, MA). Data were reported in pg/ml for each sample and each cytokine. To allow calculation of mean values and graphic analysis, all concentrations below the level of quantitation were set to 0.1 [2]. The mean, median, and maximum values of each cytokine for all the samples are also shown in Table 1.

The 7 mAbs and 2 controls used in our study are described in Table 2, which shows the target, manufacturer, number of samples, expected results and class for each mAb. The "Expected results" column in Table 2 is based on the clinical literature (Tocilizumab and Palivizumab) and on the mechanism of action of the research grade mAb being similar to a compound that has clinical results (for Anti-CD28 SA, Anti-CD80, Anti-CD22, Anti-IL-1, or Anti-IL-5) [30]. The "class" column is based on the expected reaction where severe CRS is caused by Anti-CD28 SA; no infusion reactions have been reported for the remaining treatments. The data were thus grouped into two categories, "CD28" and "Safe." The "CD28" class contained samples only from cultures treated with Anti-CD28 SA. The "Safe" class contained mAbs that are not likely to cause CRS or an infusion reaction, and controls.

The data set analyzed in this paper contains a total of 432 samples that were measured through 11 runs of the assay. The information for each run is shown in Table 3, including donors in each run, treatments used in each run, number of samples per treatment, and total number of samples for each run. The sizes of sample sets corresponding to different treatments are uneven, an observation that would affect the performance of subsequent analyses.

2.2. Hierarchical Cluster Analysis (HCA)

We will compare and integrate automatic methods to analyze and separate mAbs and controls based on the known CRS responses of the

Table 1
List of cytokines measured in the assay (the minimum values of all 11 cytokines measured were below the level of quantitation. We use 0.1 pg/ml as minimum value for all cytokines.).

Cytokine	Mean (pg/ml)	Median (pg/ml)	Maximum (pg/ml)
IL-1β	240.8	140.4	1932.3
IL-2	12.0	2.3	406.2
IL-4	2.2	0.5	45.2
IL-6	4897.6	1620.7	39,195.5
IL-10	6.3	2.7	100.8
IL-12(p70)	8.3	5.4	55.5
IL-17	22.1	0.1	263.2
IL-18	13.0	10.6	71.1
IFN-γ	4661.2	10.4	90,400.6
TNF-α (monomeric)	433.8	178.1	3729.6
TNF-α (trimeric)	294.4	131.8	1809.5

[2] Quorum Review IRB approved protocol #NOCOMPOUNDNAP1001.