Using Systems Biology-based Analysis Approaches to Identify Mechanistically Significant Adverse Drug Reactions: Pulmonary Complications from Combined Use of Anti-TNFα Agents and Corticosteroids

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Abstract

Anti-TNF drugs are frequently associated with serious Adverse Events (AEs), which necessitates an improved understanding of individual factors that determine efficacy and safety of anti-TNF agents. We mined the US FDA's Adverse Event Reporting System (AERS) for anti-TNF-associated AEs to identify and stratify patient subgroups and drug combinations that exhibit specifically correlated complications. We demonstrate the existence of patient subgroup and anti-TNF agent-specific associations for relative risks of developing known and novel AEs including infections, edema, and organ damage associated processes. Concomitant use of anti-TNFs with corticosteroids significantly increased risk of AEs (p < 0.001) including pulmonary fibrosis and pulmonary edema. Using these tightly correlated phenotypes, we mined mouse phenotype data to identify the molecular basis of these AEs. Multiple pathways and networks that regulate injury response, fluid regulation, and wound healing were implicated suggesting modification of anti-TNF-based therapeutic strategies to minimize corticosteroid-based combinatorial risk of severe AEs.

Introduction

Tumor Necrosis Factor (TNF), a pro-inflammatory cytokine produced by the macrophages and lymphocytes, plays a major role in the cascade activity triggered in the human immune response. It has been reported that TNF can induce pulmonary edema by means of augmenting reactive oxygen species[1], which have been shown to be able to disrupt pulmonary endothelial barrier[2] and to decrease the Na^+ channel activity[3,4]. Patients with pulmonary fibrosis and idiopathic pulmonary fibrosis show elevated levels of TNF and these phenotypes are also observed in mice with overexpressed TNF[5,6]. Clinical trials aimed at suppressing these inflammatory mediators suggest that anti-TNFs may be beneficial in the treatment of pulmonary fibrosis[7]. Anti-TNF medications, usually prescribed with methotrexate or corticosteroids, are significantly effective in alleviating the symptoms of TNF-Responsive Inflammatory and Autoimmune Disorders (TRIADs) like rheumatoid arthritis, ankylosing spondylitis, juvenile idiopathic arthritis, psoriasis and psoriatic arthritis, and slowing the progression of joint damage[8]. Though the success of biologics targeting TNFα has dramatically raised therapeutic expectations in TRIADs, there are serious AEs associated with anti-TNFs and concomitant medications such as methotrexate and corticosteroids[8,9]. Due to patient sensitivity to tapering of medication, corticosteroids may be difficult to discontinue and are associated with various AEs including weight gain, increased blood pressure, and increased blood sugar[10,11]. The significant efficacy of corticosteroids, which is a reference therapy for numerous neoplastic, immunological and allergic diseases, is frequently offset by their range of adverse effects and the prescribed use of this class of drugs in at-risk TRIADs population would further increase the possibility of complications. By means of methotrexate as a comparator, we examined the relationship between anti-TNF and corticosteroids medications in TRIADs patients for identification of known and novel AEs resulting from possible drug-drug interactions (DDI) from these class-specific agents, which may perturb or subvert gene regulatory mechanisms leading to increased risk of complications. Understanding the underlying molecular mechanisms of pharmacological interactions may suggest improved and effective therapeutic strategies targeted towards mitigating the concomitant effects of anti-TNF medications and corticosteroids.

Methods

The AERS[12] stores manually reviewed MedDRA[13](Medical Dictionary for Regulatory Activities)-coded reports received by the FDA from healthcare professionals, manufacturers and consumers from the United States and around the world. The AERS reports from 2004-2010 were mined for TRIADs indications to identify differential rates of AEs reported with the administration of anti-TNFs, corticosteroids and methotrexate in children (<14 yrs.), young adults (15-24 yrs.), adults (25-65 yrs.) and elderly (>65 yrs.). Only records indicating valid age and gender and precisely specified drugs, clinical indications and AEs were extracted for the study. Reports with any cancerous indications were excluded from the study.

We defined 3 main treatment groups for TRIADs, T (anti-TNFs only), C (corticosteroids only), M (methotrexate only) and their combinations T+C, T+M, C+M and T+C+M and one non-TRIAD group (Rx), patient reports that did not indicate intake of T, C and M in any therapeutic combination. The drugs mined were T: etanercept (Enbrel), infliximab (Remicade), adalimumab (Humira), certolizumab pegol (Cimzia) and golimumab (Simponi); C: dexamethasone, decadron, methylprednisolone, medrol, solu-medrol prednisolone, prednisone and deltasone; M: methotrexate, Mtx, Rheumatrex and Trexall. Reports indicating the selective T-cell co-stimulation modulator abatacept (Orencia) and the chimeric monoclonal antibody directed against the CD20 antigen, rituximab (Rituxan, MabThera), both prescribed in the event of primary anti-TNF or DMARD inefficacy were excluded from the study to focus on primary treatment regimen. The data-mining was conducted using *AERSMine*, (http://research.cchmc.org/aers/, manuscript under preparation), a java-based web-server on UNIX platform, that uses MySQL and Apache Lucene-based in-memory indexing of AERS drugs, indications, reactions in addition to demographic age and gender sub-groups.

The AE occurrences across the therapeutic groups were normalized against corresponding occurrences in non-TRIADs subgroups. Considering the diverse toxicity profiles of T, C and M, we adopted a 1.6x fold change as a threshold on these normalized frequencies to identify AEs that had statistically significant differences when grouped by drug-treatment using Welch ANOVA with *p*-value cutoff of 0.05 and Benjamini and Hochberg False Discovery Rate. This list of significant AEs was then hierarchically clustered using standard correlation with complete linkage to identify specifically correlated phenotypes that represented differential occurrences within each population subgroups and drug therapies. Using the tightly correlated phenotypes as identified through clustering, as a seed, we identified mouse genes and these phenotype-labeled gene lists were functionally enriched for mouse phenotypes, pathways and Gene Ontology using ToppCluster[14] (*p*-value cut-off of 0.5) to create a high-dimensional biological network representation of gene functional associations and interactions[14,15].

Results

The AERS received 1,927,191 patient-reports with non-cancer indications, which included 134,384 (6.97%) patients with TRIADs and 1,792,807 (93.3%) patients with non-TRIADs indications. A 1-way ANOVA with a *p*-value cutoff of 0.05 identified 301 statistically significant AEs across adults and elderly subgroups when grouped by drug therapy. These AEs were selected from a list of 495 significant AEs with reporting rates of at least one report per thousand and included pulmonary edema, fibrosis, interstitial lung disease, pleural effusions and infections among various other complications that were significantly higher ($p < 0.001$) in patients on corticosteroids. The occurrence of AEs in T cohorts were relatively lower than C and T+C therapy groups suggesting an increased risk of serious complications with concomitant corticosteroids ($p < 0.0001$) compared to methotrexate. This is particularly important in view of the treatment regimen, which advocates the concomitant use of corticosteroid therapy until the effect of the TNF-antagonists is clinically observed[8]. This prescribed intake of anti-TNFs with corticosteroids for TRIADs symptoms necessitates the need to investigate the risks of concomitant medications that may result in severe and life-threatening complications. The adverse effect of the pharmacological interactions of T+C translated to significant risk increase (at least 1.5-fold) of pulmonary fibrosis (2.5), interstitial lung disease (1.9), sepsis (2.3), septic shock (1.8), pulmonary edema (2.5) and pneumonia (1.6) within the T+C cohorts in comparison with T+M therapy group. The risks of interstitial lung diseases, sepsis, pneumonia, pulmonary edema and other serious AEs were also elevated in the elderly T+C subgroups that were at most risk of life threatening adverse effects (table 1). The relative risks increase (RRI) or reduction (RRR) for AEs varied in the adult and elderly sub-groups on T+M and T+C (fig. 1b, only elderly males shown). The TRIADs indications are characterized by elevated levels of pro-inflammatory cytokines, especially TNFα, known for its role in the pathogenesis of autoimmune and inflammatory disorders, host defense mechanisms and initiating response to local injury. However, in excess, TNFα leads to inappropriate inflammation and consequent tissue damage[16], which may explain the increased probability of tissue injury in patients with autoimmune and immunoinflammatory disorders.

Biological network representation of gene functional associations and interactions

The highly correlated phenotypes, pulmonary edema and fibrosis, were used as a seed to identify mouse genes[15,17] whose knockout conferred similar phenotypes, which along with the TNF and glucocorticoid-regulated co-expressed genesets[15,18] were comparatively enriched for mouse phenotypes, Gene Ontology and pathways through ToppCluster[14], a multiple gene list feature analyzer. The functionally enriched matrix was then visualized in Gephi[19] to represent significant functional terms by color codes and node-sizes. The visualization partitioned out gene lists to show distinct functional separations with pulmonary fibrosis and corticosteroids significantly enriched for sets of genes that confer specific phenotypes such as impaired and delayed wound healing and also enriched in hypoxia-

inducible factor – 1α (HIF-1 α) and fibrinolysis pathways. TNF up-regulated and glucocorticoid down-regulated genes were significantly enriched for TLR, VEGFR pathways, associated with regulation of various biological processes and their knockout conferred phenotype of enhanced wound healing.

(a)

(b)
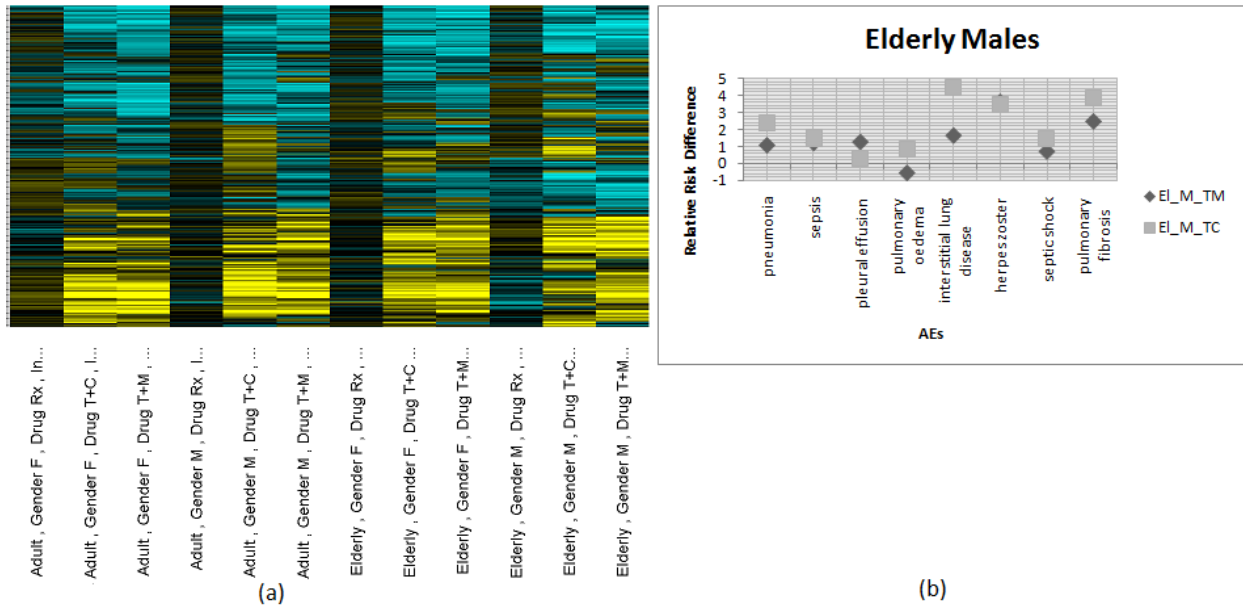
Figure 1. (a) The comparative rates of AE occurrences in T+C and T+M appeared to indicate risk increase with C intake, which was not observed with concomitant methotrexate, Rx = non-TRIADs **(b)** By means of the occurrences in non-TRAIDs demographics as the control event rate (CER) and occurrences in the T+M and T+C groups as exposed event rate (EER), we calculated the RRI (>0) or RRR (<0) for each AE using (EER-CER)/CER. Pneumonia, interstitial lung disease, pulmonary edema, pulmonary fibrosis exhibited RRI in the T+C demographics compared to T+M.

Table 1. The relative risks of AEs across treatment cohorts as identified by differential rates of occurrences and demonstrated by hierarchical clustering. The p-values, calculated using a Chi-Squared test with correlated occurrences of these AEs in non-TRIADs, suggest a statistical significance in the relative risks across the cohorts. T+C appeared to present an increased risk of life-threatening AEs in comparison with T+M.

		Relative Risks – All Patient Records, all *p-values* < 0.001			
AEs	**M (no. of records)**	**C**	**T**	**T+M**	**T+C**
pulmonary fibrosis	7.0878 (27)	3.103 (8)	1.5597 (136)	3.1195 (99)	7.8173 (72)
interstitial lung disease	7.6978 (75)	16.2268 (107)	0.84298 (188)	2.9075 (236)	5.5185 (130)
pulmonary edema	1.0486 (15)	3.0987 (30)	0.42755 (140)	0.70483 (84)	1.7926 (62)
pneumonia	2.1476 (124)	2.9429 (115)	1.1403 (1507)	1.8461 (888)	3.0301 (423)
sepsis	3.3805 (88)	2.2137 (39)	0.74683 (445)	1.3741 (298)	3.0983 (195)
pleural effusion	2.9826 (50)	2.4679 (28)	0.79484 (305)	1.6826 (235)	1.9984 (81)
Total records in cohorts	**3425**	**2318**	**78398**	**28534**	**8281**

Dysregulated injury response and wound repair in pulmonary edema and fibrosis

The functional enrichment map (fig. 2) showed that edema/fibrosis networks were deeply associated with TLR/TNFα-associated signaling pathways and extensively intersected with glucocorticoid-affected pathways regulating injury response, fluid regulation and wound healing. In TRIADs patients, the elevated levels of pro-inflammatory mediators can lead to tissue injury while increasing the expression of SERPINE1. The increase in the expression of SERPINE1 by TNFα is demonstrated in the co-expressed geneset, which also shows up-regulation of SERPINE1 by corticosteroids. The treatment cohorts showed increased rates of occurrences of pulmonary edema, fibrosis and interstitial lung disease in patients on corticosteroids, which may suggest a mechanistic link between increased expression of SERPINE1 and these pulmonary phenotypes and possibly implicating the role of

SERPINE1 in the pathogenesis of these pulmonary manifestations. The gene-level network (fig. 2) shows that TRIADs patients are at an increased risk of lung disorders due to the elevated levels of pro-inflammatory cytokines and this risk is potentially amplified by the use of corticosteroids due to up-regulation of SERPINE1 (table 1). The patients on anti-TNFs may induce a TNF-blockade mechanism that down regulates the SERPINE1 expression and promoting wound healing via positive regulation of vascular angiogenesis. The up-regulated expression of SERPINE1 by the pro-inflammatory cytokines negatively regulates wound healing, blood coagulation and fibrinolysis and could potentially lead to increased risk of tissue injury, connective tissue damage, delayed wound healing, pulmonary edema and fibrosis.
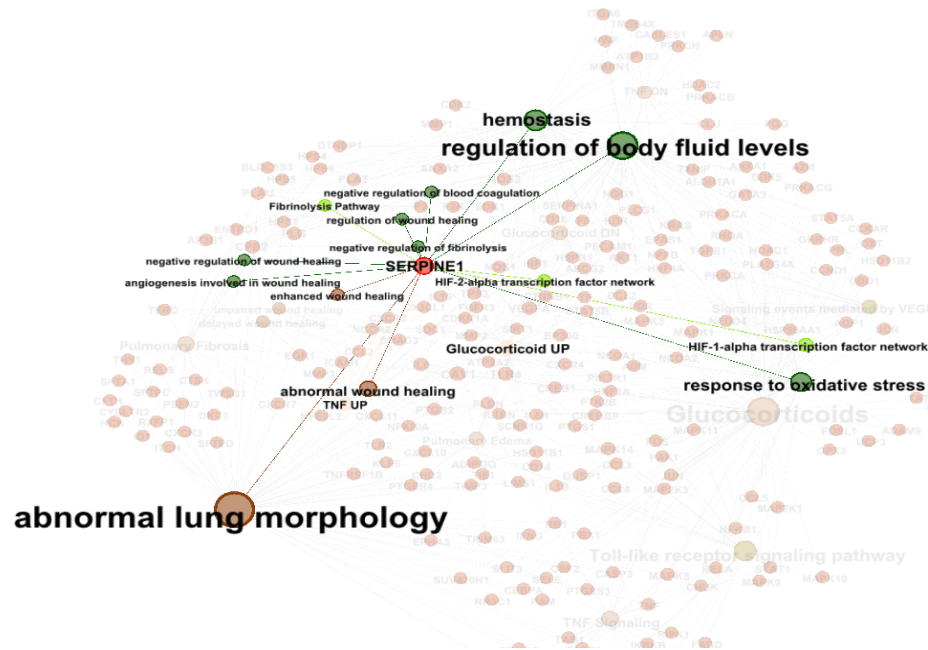
Figure 2. Plasminogen activator inhibitor-1 (SERPINE1) is a protein encoded by the SERPINE1 gene. SERPINE1 is a serine protease inhibitor that inhibits tissue plasminogen activator (tPA) and urokinase (uPA), activators of plasminogen and hence fibrinolysis. SERPINE1 plays an important role in wound healing and repair processes as it negatively regulates fibrinolysis and blood coagulation while SERPINE1 knockout mice have shown enhanced wound healing.

Increase in the expression of SERPINE1 inhibits wound healing, which is necessary to suppress pulmonary edema, pulmonary fibrosis and interstitial lung diseases. The co-expressed genesets show SERPINE1 is up-regulated by TNF and corticosteroids, which impairs the fibrinolytic activity and affects vascular angiogenesis and tissue remodeling leading to delayed or impaired wound healing. The downstream effects of SERPINE1 inhibit the activation of prostaglandin E2 and COX-2, which exhibit potent anti-fibrotic activity in the lung[20]. TNF-blockade down-regulates the expression of SERPINE1, which is augmented by the concomitant effect of corticosteroids. The reduced expression of SERPINE1 may lower the risk of pulmonary diseases and tissue injuries as seen in anti-TNF monotherapy patients (table 1). This subverted and perturbed nature of the SERPINE1 by anti-TNFs and corticosteroids may explain the increased risk of edema/fibrosis in TRIADs patients and makes it a good candidate for therapeutic evaluation.

Discussion

The fibrinolytic system is one of the key endogenous defense mechanisms whose activity is in plasma is largely determined by the balance between tPA and its natural fast- acting inhibitor, SERPINE1[21]. Local plasminogen activation is impaired in tissues where SERPINE1 is overproduced, which profoundly affects vascular angiogenesis and tissue remodeling[22,23]. This impaired fibrinolytic activity of the lung is a common manifestation of acute and chronic inflammatory pulmonary disorders. The fibrinolytic system is active during repair processes, which facilitate normal injury response and wound healing that restore injured tissues to normal, and the upregulation of SERPINE1 reduces fibrinolysis causing delayed and abnormal would healing progressively leading to pulmonary fibrosis. The direct relationship between SERPINE1 expression and the extent of pulmonary fibrosis following lung injury

suggests that human diseases, including pulmonary fibrosis and idiopathic pulmonary fibrosis, that generate fibrotic responses in pulmonary tissue, are good therapeutic candidates for the SERPINE1 antagonist treatment[21,24–26].

The deleterious effects of the elevated levels of pro-inflammatory mediators expose the TRIADs patients to increased risk of pulmonary ailments further complicated by the autoimmune nature of the disease. These cytokines play an important role in normal injury response and their early induction is crucial to regulation of wound healing. Glucocorticoid-treated mice have shown severe defects in wound healing, delayed wound re-epithelialization and impaired granulation tissue formation. Corticosteroids significantly reduced the normal induction of these cytokine expressions demonstrating that impaired expression of these factors is associated with wound healing defects[27]. These deleterious effects of corticosteroids may be due to a direct inhibitory effect on genes regulating the vascular angiogenesis but also due to suppression of inflammatory phase of healing by inhibiting leukocyte and macrophage activation and infiltration. The reduced expression of pro-inflammatory cytokines is due to the reduced invasion of cytokine-producing inflammatory cells into the wound and inhibition of cytokine expression by different cells in the wound, which leads to reduced growth factor expression which is required for normal wound healing[27]. The concomitant intake of corticosteroids could potentially subvert the positive regulation of injury response and wound healing initiated by anti-TNFs leading to increase occurrences of pulmonary AEs.

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