TNF Inhibitor Use and TNF Receptor 2 Levels Above the Measurable Range in RA

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Introduction

- Tumor Necrosis Factor Receptor II (TNFR2) is increasingly used to study cardiovascular disease (CVD) in diverse patient populations
- TNF inhibitors (TNFi) are a common treatment for inflammatory conditions, such as rheumatoid arthritis (RA)
- Whether TNFi's interfere with TNFR2 assays is not known

Objective

- To determine whether TNFi's interfere with TNFR2 measurements
  - Etanercept is a fusion protein with a TNF receptor derived from DNA sequence that codes for human soluble TNFR2
- Hypothesis: etanercept binds to the monoclonal TNFR2 antibody and impacts measured TNFR2 levels in patients using etanercept (Figure 1)

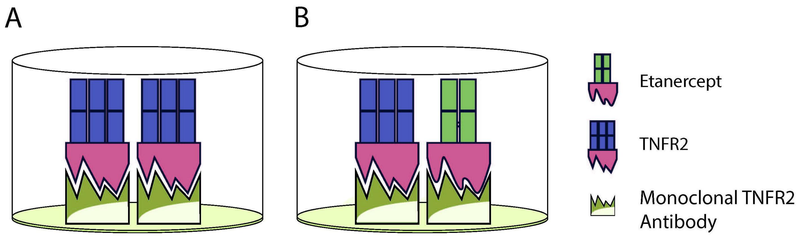

Figure 1. Illustrative example of ELISA showing A) expected TNFR2 binding B) hypothesized binding, including etanercept to the monoclonal TNFR2 antibody. TNFR2 and etanercept share a similar soluble extracellular TNFR2 portion (in pink), recognized by the monoclonal TNFR2 antibody

Methods

- Subjects were from Brigham and Women's Hospital Rheumatoid Arthritis Sequential Study (BRASS) registry
  - Clinical data including disease activity and treatment collected
  - High sensitivity-C-reactive protein (hs-CRP) measured annually
- TNFR2 was measured using a commercial ELISA kit (R&D Systems, Minneapolis, MN)

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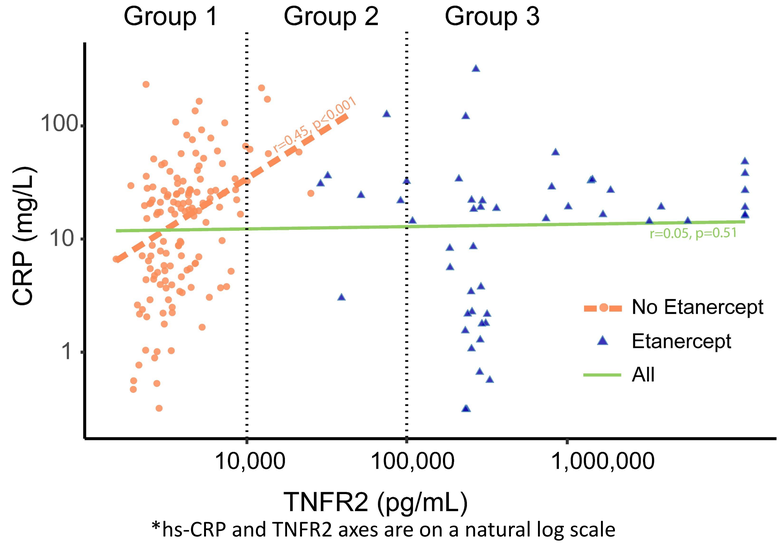
BRASS Brigham and Women's Hospital Rheumatoid Arthritis Sequential Study.
BRASS receives support from: Crescendo Bioscience, Inc; Bristol Myers Squibb; UCB; Sanofi

Methods

- TNFR2 levels categorized into 3 groups:
  1) Typical Range (>46 and ≤10,000 pg/mL)
  2) Requiring 200-fold dilution (>10,000 and ≤100,000 pg/mL)
  3) Levels exceeding measurable values (>100,000 pg/mL)
- Spearman correlation between hs-CRP and TNFR2 calculated for:
  - All subjects
  - All subjects, excluding those on adalimumab, etanercept, or infliximab individually
- Separately, a solution containing only etanercept was analyzed directly by a TNFR2 ELISA kit in increasing dilutions

Results

- 190 RA subjects, mean age 60 years, 84% female, 73% anti-CCP positive and hs-CRP ranged from 0.31-310 mg/L
- TNFi use at time of blood draw: etanercept 50 (26%), adalimumab 25 (14%), infliximab 15 (8%), certolizumab 1 (.5%), golimumab 0
- All subjects with TNFR2 level exceeding measurable level (Group 3) were on etanercept therapy (Figure 2)

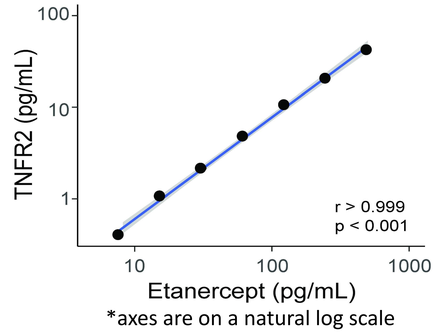


*hs-CRP and TNFR2 axes are on a natural log scale

Figure 2. Influence of etanercept on correlation between hs-CRP and TNFR2

Results

- No significant correlation found between hs-CRP and TNFR2 for all subjects (green line). After excluding subjects on etanercept, the correlation was significant, r=0.45, p<0.001 (orange line) (Figure 2)
- After excluding patients on adalimumab or infliximab, there were no significant changes in the correlation between hs-CRP and TNFR2 (data not shown)
- A $1x10^7$- fold dilution of pure etanercept was required to obtain TNFR2 levels within the calibration curve, displaying a linear relationship with approximately 10% cross-reactivity (Figure 3)



*axes are on a natural log scale

Figure 3. Correlation between etanercept and ELISA for TNFR2 detection

Strengths/Limitations

- First study to investigate potential cross-reactivity between TNFi and TNFR2 assay
- Study tested etanercept solution to show direct binding
- Too few patients to study potential cross-reactivity with certolizumab or golimumab

Conclusions

- Etanercept use interferes with measurement of TNFR2 levels
- Cross-reactivity was specific to etanercept and not adalimumab or infliximab
- Studies using TNFR2 should be carefully designed in populations where etanercept is used as treatment