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TITLE: Immunogenicity testing for TNF antagonists in the clinical laboratory

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Slide 1:

Hello, my name is <**Eszter Lazar-Molnar**>. I am an <**assistant professor of Pathology at the University of Utah School of Medicine, and medical director in immunology at ARUP Laboratories**>. Welcome to this Pearl of Laboratory Medicine on "**Immunogenicity testing for TNF antagonists in the clinical laboratory**."

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Tumor necrosis factor alpha, or TNF is a pro-inflammatory cytokine that plays central role in immunity and inflammation, and in the pathogenesis of various autoimmune and inflammatory diseases.

Monoclonal antibody therapeutics targeting TNF have revolutionized the treatment of patients suffering of various autoimmune and chronic inflammatory diseases.

TNF antagonists are a class of biologics that are increasingly used worldwide.

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Currently there are 8 TNF antagonist drugs approved in the US. Structurally, most of them are monoclonal antibodies, such as infliximab, which is a chimeric mouse-human antibody; adalimumab and golimumab, which are fully human antibodies; and certolizumab which is a Fab fragment of a monoclonal antibody conjugated to pegol to increase half-life. To a variable extent, these antibody drugs may contain non-human amino acid sequences, shown with green color on this slide. Etanercept is structurally different from the other drugs, since it is engineered as a fusion protein between TNF receptor 2, and the Fc part of human IgG1, and it binds not only to TNF but also to lymphotoxin alpha.

In 2016 biosimilars to 3 of these drugs (infliximab, adalimumab and etanercept) were approved, marked with red asterisks on this slide. A biosimilar is a biological product that is highly similar structurally and functionally to an existing FDA-approved reference product, and has no clinically meaningful differences from the reference product in terms of safety and effectiveness.

TNF antagonists constitute a significant share of the global pharmaceutical sales market. 3 of the five original drugs were within the top five most selling drugs for 2016, Adalimumab being #1, followed by etanercept at #3 and infliximab at #5, totaling over $26 billion dollars combined, from the total of $450 billion pharmaceutical sales in the US last year.

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TNF antagonists are approved for the treatment of various autoimmune and chronic inflammatory diseases. This table shows clinical indications for each of the generic drugs; the year of first FDA approval shown. They are widely used in rheumatology for the treatment of rheumatoid arthritis, psoriatic arthritis and ankylosing spondylitis; some are also used in juvenile idiopathic arthritis. In gastroenterology they are used for the treatment of inflammatory bowel disease including Crohn's disease and ulcerative colitis. Dermatological uses include plaque psoriasis for infliximab, etanercept and adalimumab.

TNF antagonists dramatically lower disease activity and induce remission in the majority of patients suffering of these diseases ("miracle" drugs).

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However, the use of TNF antagonists confronted clinicians with major challenges, such as adverse effects due to immunosuppression: tuberculosis reactivation, sepsis, opportunistic infections or malignancies, which are due to the crucial role of TNF in these mechanisms.

Another major challenge is the development of treatment failure, which affects up to 60-70% of the patients. Treatment failure can be primary, or secondary, with various underlying mechanisms.

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Primary treatment failure indicates lack of improvement of clinical signs and symptoms during the initial induction therapy, which affects about one third of the patients. Primary treatment failure is mostly due to pharmacokinetic issues, leading to increased drug clearance and consequently low drug concentrations at the site of inflammation. Factors associated with primary response failure include high baseline TNF, which correlates with increased CRP levels, low albumin, large body size and male gender.

Primary treatment failure is managed by intensification of therapy by increasing dosage, or shortening dosing interval.

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Secondary treatment failure indicates loss of clinical response after initial improvement of clinical signs and symptoms, and occurs in about half the patients previously responding to the drug.

Secondary treatment failure is predominantly caused by immunogenicity of these protein based drugs, and the production of anti-drug antibodies (ADA) during treatment.

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Immunogenicity is elicited by repeated administration of exogenous proteins, for example vaccines, or other protein-based therapeutics. Repeated injections of protein, or antibody-based drugs such as TNF antagonists elicit the formation of anti-drug antibodies over time. Immunogenicity is affected by treatment related factors, such as 'foreignness' of the drug, such as the presence of exogenous sequences, route of administration (iv vs sc), number of doses, length of treatment. Patient related factors include genetic background (HLA), presence of inflammatory cytokines that may skew T cell responses, and others.

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Studies have shown that the appearance of ADA is associated with a significantly higher risk of secondary treatment failure, and the appearance of ADA correlates with decrease of serum drug levels over time. This slide shows increasing incidence of ADA following repeated iv IFX treatment at 0, 2, 6 and then every 8 weeks in a cohort of rheumatoid arthritis patients, measured at 1.5, 3 and 6 months of treatment. 44% of the patients were ADA positive at 6 months of therapy, showing decreasing trough drug levels.

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Clinical management of patients with treatment failure to TNF antagonists has been largely empirical, based on clinical outcomes. Interventions included intensification of treatment by increasing the dose, or shortening dose intervals; concomitant administration of immunosuppressives such as metothrexate, or changing to a different type of TNF antagonist, and if everything else failed, changing to an entirely different class of drug. In the empirical approach, no attempt was made to identify the cause, or the mechanism of treatment failure.

A test-guided approach for clinical management involves the use of laboratory testing to measure serum drug levels, and detect the presence of anti-drug antibodies, and select treatment based on the most likely mechanism of treatment failure. This approach minimizes adverse effects caused by immune complexes between drug and ADA. Of interest, a randomized controlled trial performed in Europe in Crohn's disease patients supported the cost-benefit of test-guided management over empirical strategy in patients who developed treatment failure to TNF antagonists. Individualized test-guided strategy significantly reduced average treatment costs per patient by about 50%, compared to the empirical approach, without differences in clinical efficacy.

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Testing for drug levels and detecting immunogenicity to TNF antagonists is one of the fastest growing areas in the clinical laboratory.

Several different methodologies are currently used for measuring serum drug levels, and detecting anti-drug antibodies. Most of the testing platforms employ binding-based methods, solid phase binding such as traditional sandwich ELISA, and the more commonly used bridging ELISA. Liquid phase binding includes the HPLC-based homogenous mobility-shift assay (HMSA), which uses fluorescent labeling, followed by HPLC-based separation of bound complexes. Additional methodologies include LC-MS/MS for measuring drug levels, based on identification of clonotypic peptides obtained by trypsin digestion of the drug. Because of the trypsin digestion step the LC-MS/MS method detects both free drug, and drug complexed with ADA. Functional assays such as a cell-based reporter gene assay is also available, which measures the ability of the drug to block TNF receptor signaling, providing a direct functional readout of drug activity.

Importantly, methods for measuring drug levels perform and compare well. Several studies, including our group, confirmed strong correlations for drug levels measured by different assays, despite of differences in the analytical platforms performed in different laboratories.

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Methods for measuring anti-drug antibodies face multiple challenges, such as the complexity of measuring antibodies against antibodies in the presence of high levels of endogenous immunoglobulins.

Another common challenge is that patients that are on therapy will present with interfering serum drug levels, which complicates the detection of anti-drug antibodies because it leads to the formation of immune complexes unavailable for detection by most assays. To minimize drug interference, anti-drug antibodies should be measured at drug trough levels (right before the next dose, when the serum drug level is at minimum).

A third challenge is that most assays are unable to differentiate between binding and function neutralizing anti-drug antibodies. Antibodies are relatively large molecules, around 250kD, immunogenicity may be directed against various parts of the molecule, and not all anti-drug antibodies may block the idiotype, or active site, which mediates the functional effect of the drug.

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Binding assays are affordable and easy to use, however, they can give false positive results due to aggregation of molecules on the solid phase. Bridging ELISA takes advantage of the bivalency of antibodies, which allows for the formation of a bridge between plastic immobilized drug, ADAs from the patient's serum and enzymatically labeled drug used for detection. However, false positive results may occur due to the

presence of interfering antibodies such as rheumatoid factor, or anti-idiotype antibodies, which bind to IgG non-specifically. False negative results may occur due to drug interference when the drug is present at high concentrations in the patient's serum and the excess drug prevents bridge formation. Furthermore, this method is unable to detect IgG4 isotype, because IgG4 is monovalent, and bispecific.

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The homogenous mobility-shift assay detects ADAs by complex formation with fluorescently labeled drug molecules added to the serum, followed by size-exclusion HPLC separation of antibody-bound, versus free labeled drug. HMSA detects any antibody that is binds to the drug, including functional, neutralizing, and non-neutralizing antibodies.

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The functional reporter gene assay uses cells carrying a TNF-inducible firefly luciferase reporter gene construct, which turns on by TNF receptor signaling, resulting in luciferase expression, and generation of bioluminescence. To control for serum matrix effects affecting cell viability, the signal is normalized to a second, constitutively expressed Renilla luciferase signal carried within the same cell.

For measuring drug activity, the serum is mixed with TNF and incubated with the cells. The presence of drug in the serum blocks TNF activity, decreasing luminescence. For the detection of neutralizing anti-drug antibodies, the patient serum is spiked first with a fixed amount of drug. If the patient has no neutralizing antibodies in the serum, then the added drug will inactivate the TNF in the assay and there will be no TNF-induced luminescence. If neutralizing antibodies to the drug are present, they will neutralize the drug added to the assay, and TNF will be available to bind to the cells and induce TNFR mediated luminescence. The amount of neutralizing antibody in the serum will be directly proportional with the luminescence. The ADA is quantified by testing serial dilutions of the serum and identifying the highest dilution at which blocking of drug activity is no longer observed.

The RGA will only detect ADAs that are neutralizing the effect of the drug by directly interfering with TNF receptor signaling. Non-neutralizing antibodies that do not interfere with drug activity will not be detected.

As with the other assays, detection of ADA by RGA is sensitive to drug interference: the presence of drug in the serum will prevent the detection of ADA by the assay, therefore it is important to test for ADA when drug concentrations are the lowest, at trough level.

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Neutralizing, versus non-neutralizing antibodies have different mechanisms in treatment failure. Neutralizing antibodies directly interfere with the biological effect of the drug,

providing a direct explanation for the cause of treatment failure. Binding, but non-neutralizing antibodies may be transient or have no direct impact on treatment response, because they do not block the biological activity of the drug. However, non-neutralizing antibodies may alter pharmacokinetics by inducing clearance of the drug through immune complex formation. Combined use of binding assays along with functional tests in future studies are necessary to clarify the specific roles of neutralizing, versus non-neutralizing antibodies in the mechanism of treatment failure to TNF antagonists.

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Depending on the outcome of the result for both drug, and anti-drug antibody, patients that are presenting with treatment failure could be managed differently, depending on the test results. This proposed algorithm has been supported by clinical trials. First, if drug is not detected or at sub-therapeutic levels, and ADA are absent, the patient may benefit of treatment intensification (higher dose, more frequent administration). Second, if drug is absent, and ADA is present, this scenario is indicative of treatment failure due to immunogenicity to the drug, and there is no point in continuing therapy with the same drug since it will be bound to ADAs and cleared, or neutralized and will have no effect. In this case, switching to another drug from the same family may be beneficial since antibodies are drug-specific.

Third, if drug is present at an acceptable therapeutic level, and ADA is not detected, but the patient is still not responding to therapy, switching to another treatment may be beneficial due to the possibility of non-TNF driven disease. Fourth, if both drug and ADA are present, and the patient is not responding, it could indicate the presence of non-functional ADA, and repeating the test using neutralizing antibody assay is recommended.

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Testing for serum levels of TNF antagonists and ADA are primarily indicated for the management of patients with treatment failure.

However, therapeutic monitoring is increasingly becoming part of the clinical practice. A recent guideline from the American Gastroenterological Association recommends reactive therapeutic monitoring to guide changes in TNF antagonist therapy. The guideline suggests target trough concentrations for patients with active IBD on maintenance therapy, which is a minimum of 5 ug/ml infliximab and 7.5 ug/ml adalimumab. These recommendations are however for patients with active disease, based on limited numbers of randomized controlled trials. The guideline makes no recommendation for patients with quiescent IBD regarding proactive therapeutic monitoring.

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In summary, immunogenicity of TNF antagonists is an important therapeutic challenge and can lead to treatment failure. Multiple analytical methods are available clinically for measuring drug levels and ADA. Drug assays show good correlation and are relatively comparable with acceptable specificity, accuracy, and reproducibility between assays across different analytical platforms. In contrast, ADA assays differ in their abilities for detecting ADAs of different types, such as neutralizing and non-neutralizing; they may show different sensitivities to drug interference, and may differ in reported units (titer versus concentration) due to lack of commercially available antibody standards.

Testing for TNF antagonists and ADAs is primarily recommended in the context of therapeutic failure, but therapeutic monitoring is becoming part of routine clinical practice, guiding changes in TNF antagonist therapy.

The use of a test-guided strategy, instead of trial-error based empirical strategy contributes to safer, cheaper and more effective treatment, since therapy is tailored to the individual needs of the patient.

Slide 20: References

Slide 21: Disclosures

Slide 22: Thank You from www.TraineeCouncil.org

Thank you for joining me on this Pearl of Laboratory Medicine on "**Immunogenicity testing for TNF antagonists in the clinical laboratory**".