#P020

Serum Ustekinumab and Corresponding Anti-Ustekinumab Antibody: Analysis of over 2000 Patient Results Using Lab Developed Chemiluminescent Immunoassays (ECLIA)

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I. Introduction

Ustekinumab (UST), a monoclonal interleukin-12 and -23 antagonist, is indicated for ulcerative colitis, Crohn's disease and psoriasis. Therapeutic drug monitoring (TDM) assays to measure ustekinumab and anti-ustekinumab antibody (ADAb) concentrations are used to optimize therapeutic efficacy. Target concentrations should be based on UST pharmacokinetics and the relationship between serum UST and desired clinical and endoscopic outcomes. IM-UNITI data (n=397) suggest a target UST trough concentration of >1 ug/mL at steady state (week 24) [1]. Other smaller studies suggest week 8 and maintenance trough cutoffs of 2 and 4.5 ug/mL, respectively [2,3]. Here, 2097 patient results were analyzed. Though clinical histories are not known, these data reiterate the necessity of validated assays with highly sensitive lower limits of quantitation.

II. Methods

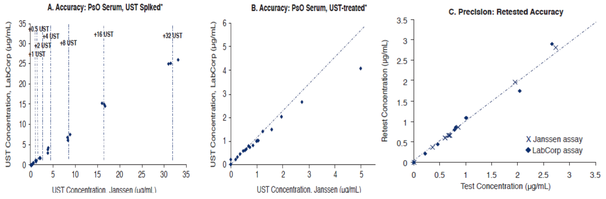
Measurements of UST drug (free, ADAb-unbound) and anti-UST antibody (total, including IgM and IgG subtypes) were performed by lab-developed electrochemiluminescence immunoassay (ECLIA) [3]. The UST drug assay is two-site immunoassay. The ADAb assay utilizes a solution phase bridging method and has demonstrated drug tolerance. All ADAb positive samples are confirmed by a signal suppression test. Precision, accuracy and lower limits of quantitation are shown in Table 1.

Table 1: Assay Characteristics

Parameters	Ustekinumab	Anti-Ustekinumab Ab
Assay Reportable Range	0.1-32+ µg/mL	40-180,000 ng/mL
Inter-Assay Precision (% CV)	<10%	<7%
Inter-Assay Accuracy (% bias)	<10%	<8%
Assay method	2-site, 2-step Immunoassay	Extraction, Solution phase bridging
Detection System	Electro-Chemiluminescence	Electro-Chemiluminescence

Both drug and ADAb ECLIAs were tested by Janssen R&D (Spring House, PA) for specificity, sensitivity, drug tolerance, accuracy, and precision and found to strongly agree with Janssen's own R&D assays used in clinical trials (Figure 1) [4].

Figure 1: Strong Agreement between UST Assays: LabCorp vs Janssen



*Lower limit of quantification = 0.16880 µg/mL for the Janssen assay and 0.12 µg/mL for the LabCorp assay.

III. Results

Of 2097 measured samples, 96 % (2012) were ADAb-free and only 4% (85) had measurable anti-ustekinumab antibodies. In the absence of ADAb, UST concentrations ranged 0.1 to >32 ug/mL (Table 2, Figure 2). Due to a sensitive lower limit of quantitation (0.1 ug/mL), only 38 (1.9%) had undetectable drug (< 0.1 ug/mL). Of all ADAb-free samples, 9.2% were < 1.0 and 24.6% were < 2.0. Interestingly, half (50.4%) had UST less than 4.0. A quarter of patient samples (26.3%) were in the range between 5.0 – 10. High UST (>10) occurred in 8.9% while a small subset of patient samples (2.1%) had very high UST >20.

Only 85 samples (4%) were positive for anti-ustekinumab antibodies, ranging 42 to 9634 ng/mL (Table 3). As a baseline reference, an ADAb-free mean drug level was determined from 2012 samples with UST between 0.1 – 30 µg/mL. Most immunogenicity (52%) was low in titer with ADAbs between 40 -99 ng/mL with a mean free UST of 4.5 ug/mL, suggesting that low titer anti-ustekinumab antibodies do not significantly depress concomitant free drug levels. On the other hand, when ADAbs are high titer (only 3 samples had ADAb > 1000), their concomitant UST drug levels were undetectable. 92.9% (79/85) of ADAb samples were <500 ng/mL.

Table 2: Serum Ustekinumab Drug Levels in Anti-Ustekinumab Antibody Negative Patients

Drug Range (µg/mL)	n	%	Mean	Median
< 0.1	38	1.9	<0.1	<0.1
0.1 - 0.9	147	7.3	0.6	0.6
1.0 - 1.9	309	15.4	1.4	1.2
2.0 - 2.9	286	14.2	2.4	2.4
3.0 - 3.9	272	13.5	3.5	3.5
4.0 - 4.9	231	11.5	4.5	4.5
5.0 - 9.9	529	26.3	6.5	6.3
10 - 15	114	5.7	12.4	12.0
16 - 20	42	2.1	17.3	17.0
21 - 29	27	1.3	23.6	24.0
> 30	17	0.8	33.5	32.0

Figure 2: Distribution of Ustekinumab Drug Levels in ADAb-Negative Sera

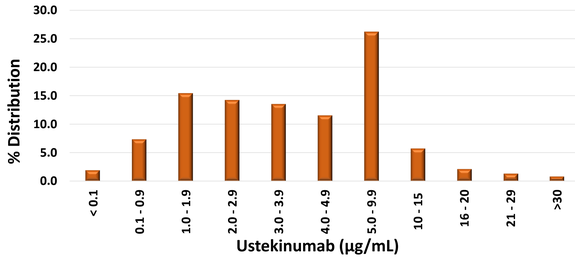


Table 3: Anti-Ustekinumab Antibody Level Distribution and Concomitant Mean Drug Levels

Anti-Ustekinumab Antibody (ng/mL)	n	Mean Drug Conc. (µg/mL)	%	% with Undetectable Drug (<0.1 µg/mL)
Undetected (<40)	2012	5.1	96% of total	0.0%
40 - 99	44	4.5	52% of ADAb positives	2.3%
100-999	38	2.3	47% of ADAb positives	11%
1000-9634	3	0.1	3.5% of ADAb positives	67%

IV. Conclusion

Here, we analyzed the UST results of 2097 patient samples. Only 4% exhibited anti-ustekinumab antibodies. There does appear to be an inverse relationship between UST and anti-UST antibody concentrations. Estimated cut-points for Low, Intermediate and High titer ADAbs are < 100, 100 - 999, and >1000 ng/mL. More than half of all observed immunogenicity (52%) was low in titer and did not significantly impact concomitant free drug. On the other hand, high titer ADAb (>1000 ng/mL), though very rare, were associated with very low or absent drug levels. Because the vast majority (93%) of detectable anti-ustekinumab antibodies were in the low to low intermediate titer range (<500), this test may be useful to detect and monitor early immunogenicity.

The therapeutic range for UST has yet to be firmly established. Nevertheless, because the therapeutic range for UST appears to be in the single digits, 1, 2 or 4 ug/mL, clinicians must be aware of the need to use an adequately sensitive UST assay. Due to this highly sensitive UST assay, undetectable drug was an uncommon (<2%) result here. More than a third (38%) of samples were within a range of 4-10 ug/mL for ustekinumab. Using target cutoffs of 1 and 2 ug/mL, 9.2% of patient samples and 24.6%, respectively, were below target. And if the target threshold of 4.0 ug/mL is used, then half (50.4%) would be considered sub-therapeutic.

Here, we demonstrate that gastroenterologists are currently using TDM to optimize UST. Better-established target concentrations for UST based on correlation to clinical and endoscopic outcomes would be beneficial to this practice of UST TDM.

V. References

1. Adedokun O et al. Gastroenter 2018;154:1660-71.
2. Soufflet N et al. J Crohns Colitis 2019;13(1):S446.
3. Battat R et al. Clin Gastroenterol Hepatol 2017;15:1427-1434.
4. Marini JC et al. Gastroenter 2019;156(3):S35.

These ustekinumab and anti-ustekinumab antibody assays constituting DoseASSURE UST™ are developed and performed at LabCorp's Esoterix specialty laboratory in Calabasas, CA.