S/N versus Titer to Quasi-Quantify Immunogenicity – Statistical Perspective

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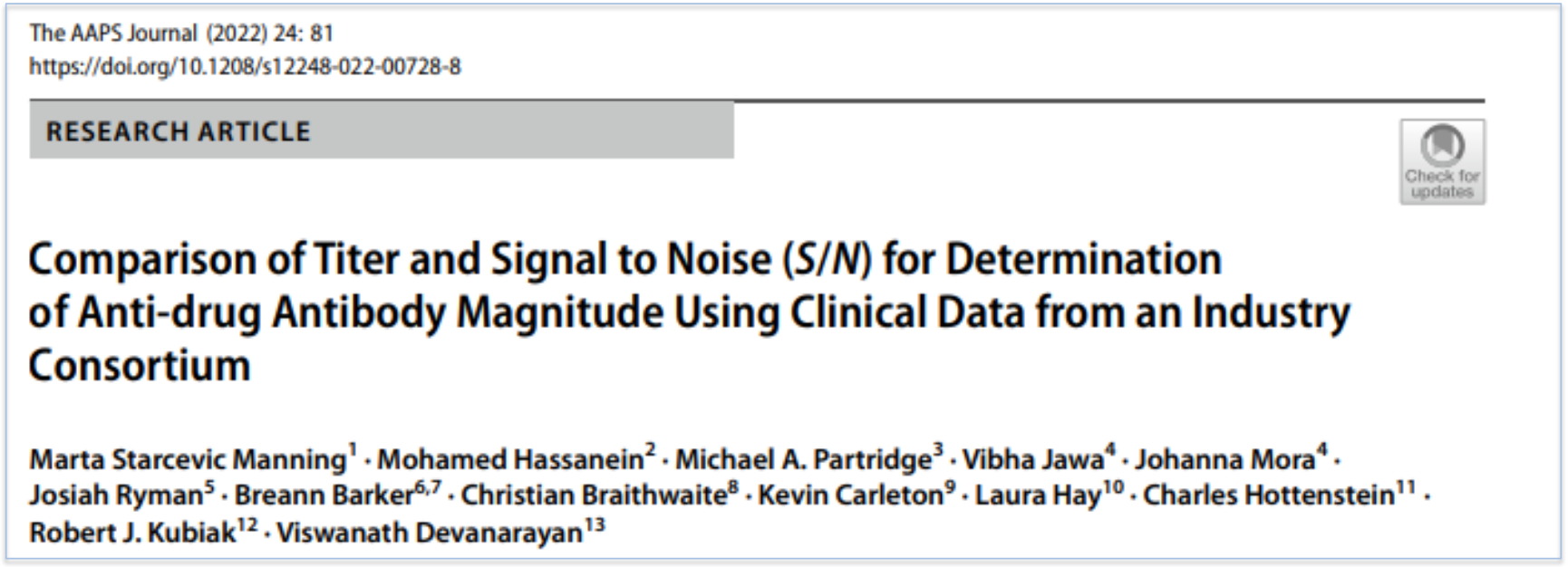
- Special thanks to Marta Starcevic Manning for leading this effort, and for contributing most of the slides/material used for this presentation.



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RESEARCH ARTICLE

Check for updates

Comparison of Titer and Signal to Noise (*S/N*) for Determination of Anti-drug Antibody Magnitude Using Clinical Data from an Industry Consortium

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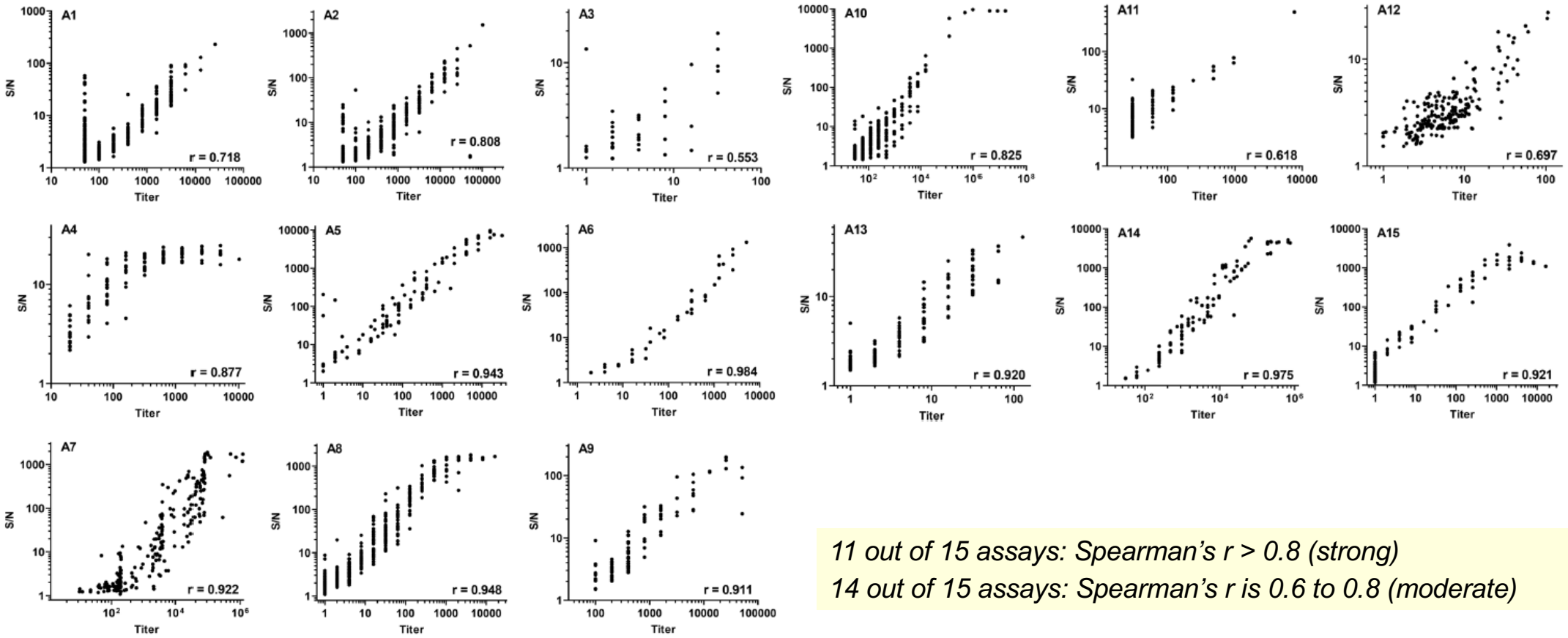
Industry Consortium: Evaluation of S/N as a Titer Alternative

- **Team of industry scientists collected:**
  - Clinical study data: S/N, Titer, PK/PD endpoints
  - Validation data: ADA and titer assay characteristics
- **Data collected (2019-2021): 15 clinical assays**
  - Therapeutic modality: 12 mAbs, 1 bispecific, 2 fusion proteins
  - Immunogenicity risk: 8 low, 5 medium, 2 high
  - Immunogenicity incidence: 2%-100%
  - Assay platforms: 12 MSD, 3 ELISAs
  - Assay formats: 13 bridging, 1 indirect, 1 SPEAD
  - Titer approach: 13 endpoint, 2 interpolated

S/N vs. Titer Correlations



11 out of 15 assays: Spearman's r > 0.8 (strong)
14 out of 15 assays: Spearman's r is 0.6 to 0.8 (moderate)

Figure-1, Starcevic Manning et al., AAPS J. 2022 Jul 12;24(4):81

Correlation of S/N and Titer versus PK/PD

Table II Correlation of *S/N* vs. Titer, and ADA Magnitude (*S/N* or Titer) vs. PK/PD

Assay number	Number of positive subjects	Number of positive samples	Spearman's rank correlation coefficient (*r*)				
			S/N vs. titer	PK vs. *S/N*	PK vs. titer	PD vs. *S/N*	PD vs. titer
A1	127	404	*0.718*	*−0.456*	*−0.286*		
A2	128	393	*0.808*			*0.400*	*0.358*
A3	30	42	*0.553*				
A4	88	119	*0.877*	*−0.182*	*−0.204*	*−0.406*	*−0.414*
A5	27	164	*0.943*				
A6	11	44	*0.984*			*−0.332*/−0.081	*−0.332*/−0.055
A7	38	238	*0.922*	*−0.776*	*−0.725*		
A8	133	499	*0.948*				
A9	49	102	*0.911*	*−0.294*	*−0.288*		
A10	249	375	*0.825*				
A11	65	111	*0.618*				
A12	80	197	*0.697*				
A13	27	147	*0.920*	*−0.348*	*−0.296*		
A14	33	117	*0.975*				
A15	46	142	*0.921*	−0.169	*−0.192*		

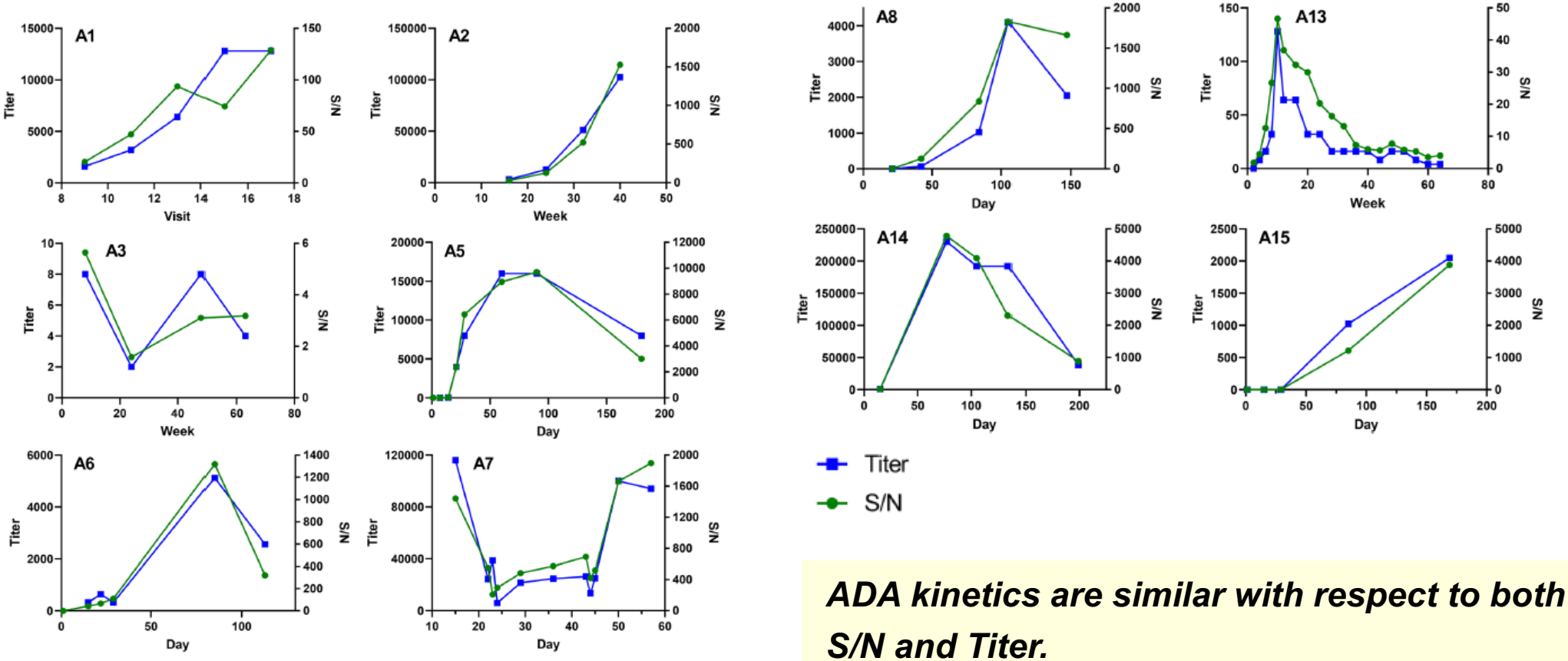
Italics indicate significant correlation values (two-tailed *p*-value <0.05). For correlation of ADA magnitude (*S/N* or titer) with PK or PD, datasets were included in the analysis if at least 50% of the positive samples had a nonzero PK or PD measurement. Assay A6 had data for two PD markers submitted

ADA anti-drug antibody, *PK* pharmacokinetics, *PD* pharmacodynamics

Except A1, S/N and Titer have comparable levels of correlations to PK and PD (p > 0.05, Hittmer's test).

Table-II, Starcevic Manning et al., AAPS J. 2022 Jul 12;24(4):81

Subject ADA Profiles



ADA profiles of the subject with the highest S/N and at least 4 positive time points was analyzed from each dataset

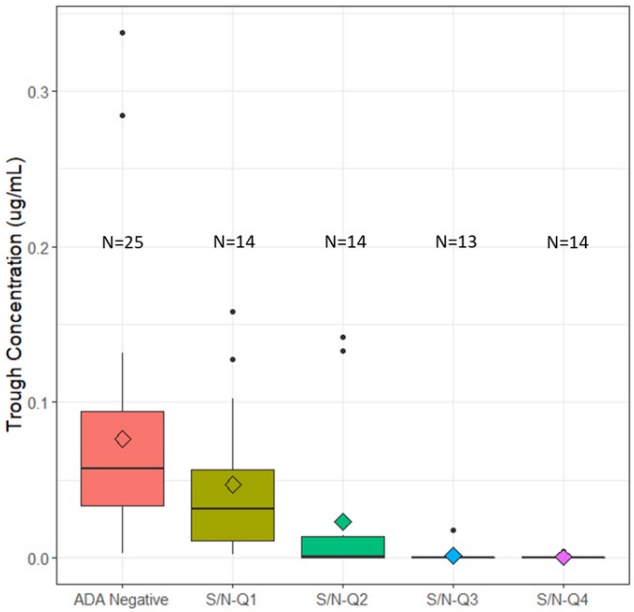
ADA kinetics are similar with respect to both S/N and Titer.

Figure 3, Starcevic Manning et al., AAPS J. 2022 Jul 12;24(4):81

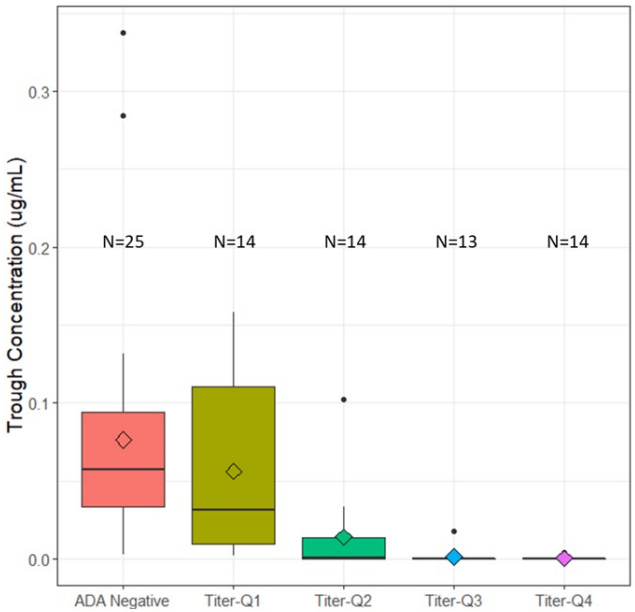
A closer look at A7

Spearman correlation		
S/N vs. Titer	PK vs. S/N	PK vs. Titer
92.2%	-77.6%	-72.5%

Distribution of dose-normalized PK trough conc. by ADA status



S/N quartiles



Titer quartiles

We took a closer look at A7 data, due to the availability of dosing information and intense PK/ADA sampling, the wide range of ADA magnitude, and the strong ADA vs. PK association

Due to nonlinearity, we evaluate the association in terms of quartiles.

The impact of ADA on PK is similar between S/N and Titer, except for slightly greater variability in the first quartile of Titers.

Figure 2, Starcevic Manning et al., AAPS J. 2022 Jul 12;24(4):81

Simulation analysis using A7 data

Impact of S/N vs Titer Correlation on clinical impact assessment

Titer vs. PK correlation is -0.73

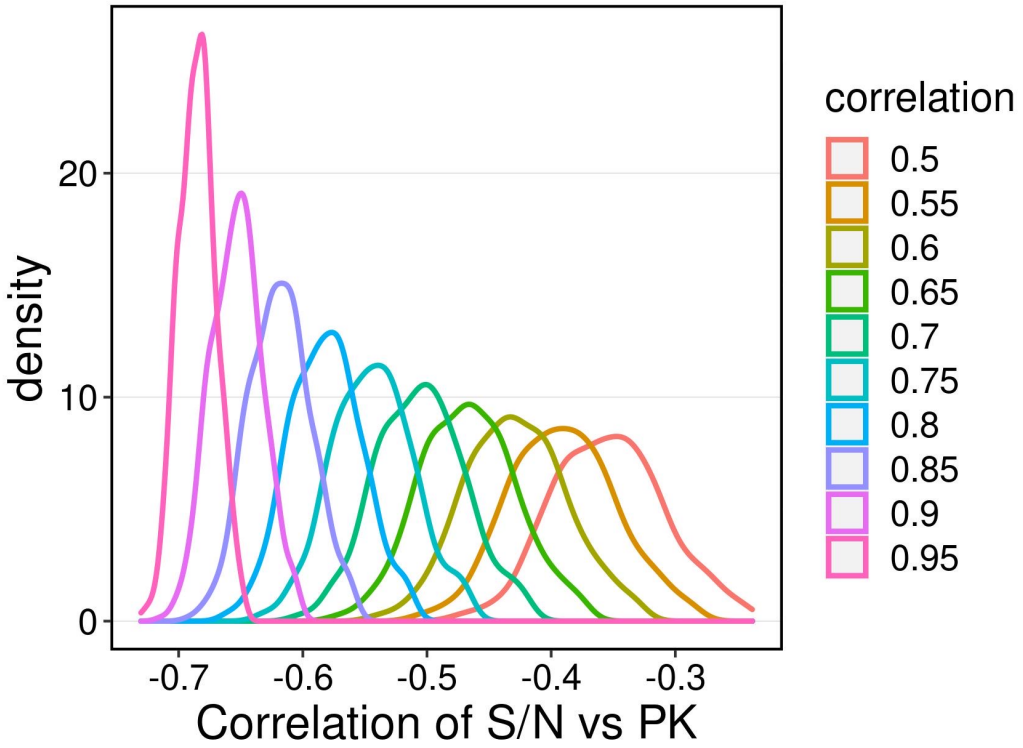
If S/N vs. Titer correlation is high, then the S/N vs. PK correlation should be close to -0.73. How high should it be?

To study further, we simulate S/N data 500 times, with correlations of S/N vs. Titer ranging from 0.5 to 0.95.

For each S/N vs. Titer correlation value, the S/N vs PK correlation is calculated, and the distribution across 500 iterations is evaluated.

We see S/N vs. PK correlation vary from -0.35 to -0.7 when the S/N vs. Titer correlation varies from 0.5 to 0.95.

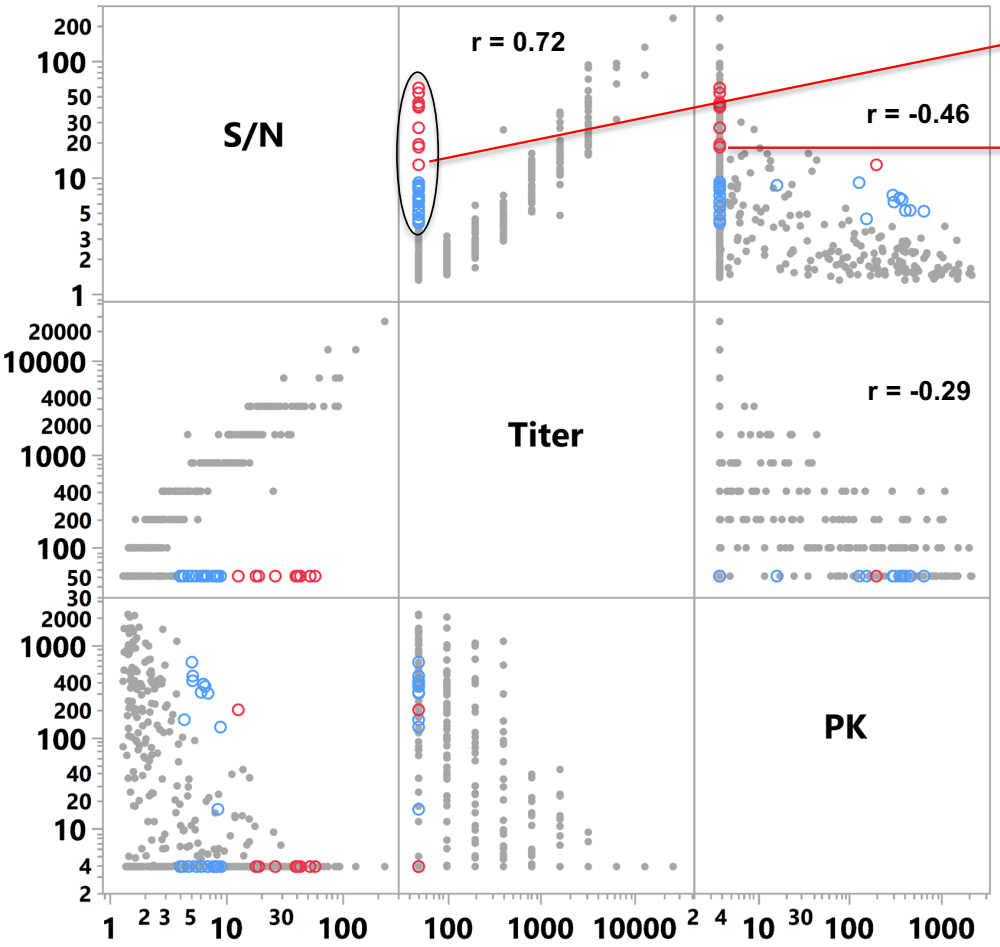
Extends to the other clinical endpoints (PD, efficacy, safety).



S/N vs. Titer correlation > 0.8 yields a similar conclusion about the clinical impact (i.e., Titer & S/N vs. PK correlation).

NB: This is conservative as it assumes Titer is the more accurate measure of ADA magnitude. Being the current standard doesn't mean it is a better measure of ADA magnitude. Titers have higher variability and do not accurately reflect certain types of ADA (e.g., low affinity/avidity).

Closer look at A1



*Samples with **high** to **moderate** **S/N and low Titer** are highlighted in red and blue, respectively.*

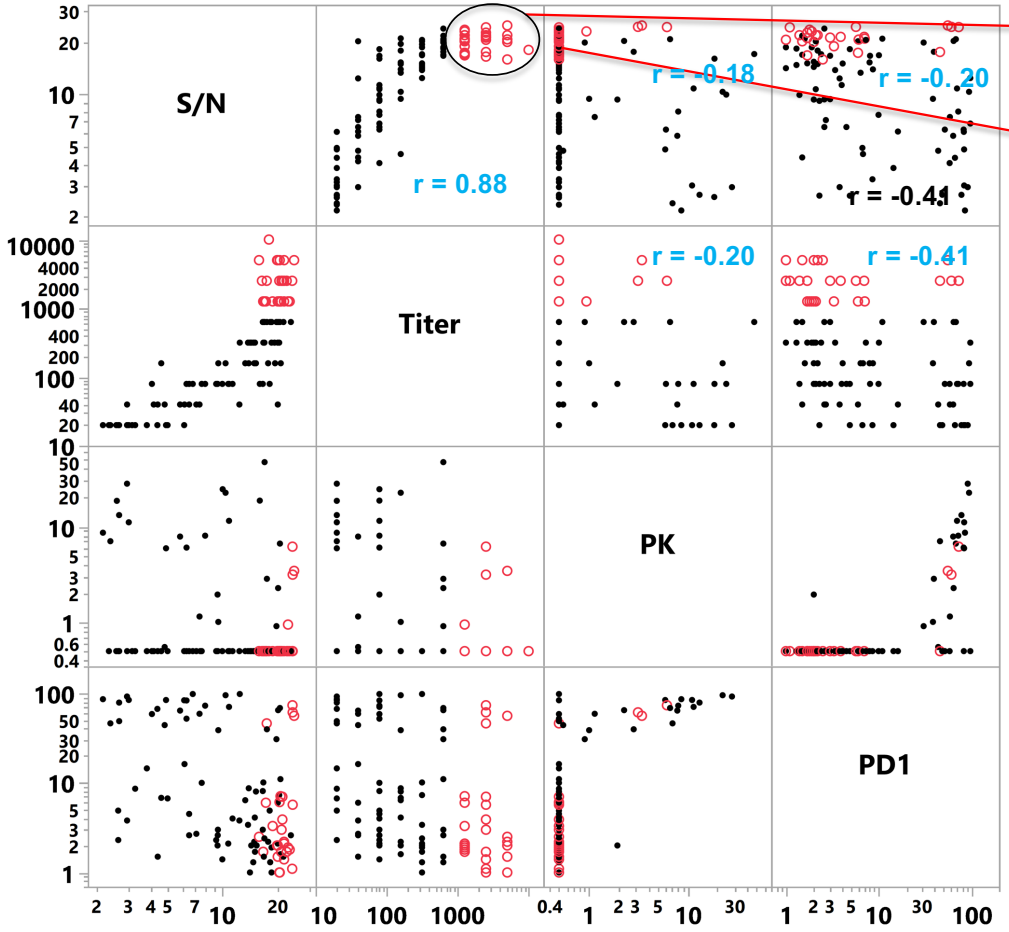
Most of the samples with high S/N and low Titer have low PK.

However, it turns out that some of these were placebo subjects. If these were treated subjects, S/N may reflect the ADA magnitude more accurately than Titer for these samples.

Furthermore, most of these samples had high pre-existing Ab.

The low Titer results could be due to the low affinity/avidity ADA that gets dissociated faster or blocked by the exogenous matrix added during titration.

Closer look at A4

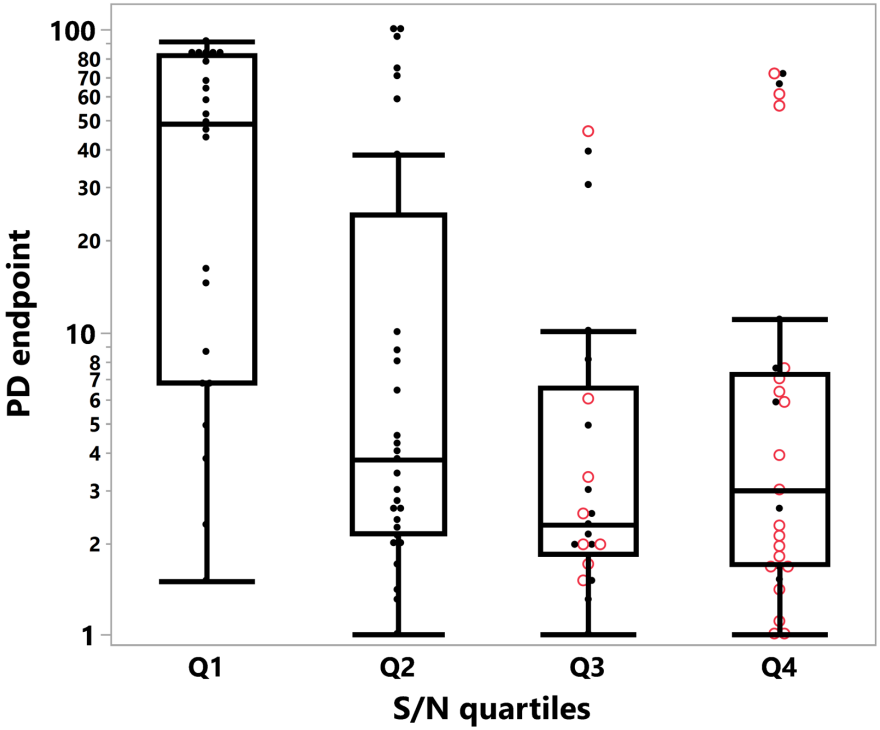


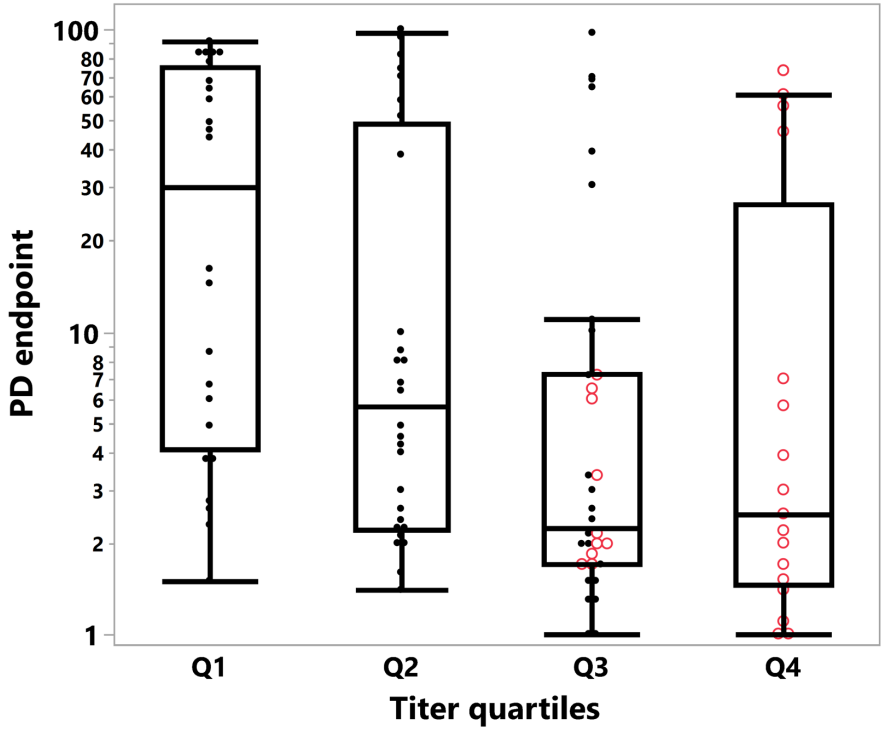
S/N plateau due to limited assay range of ELISA

Samples with S/N plateau have low PK

Although S/N might not be optimal for this ELISA with a low assay range, it doesn't compromise the impact assessment of ADA on PK and PD.

Closer look at A4 (contd.)





When assessing S/N and Titer association to PD via quartiles, the plateau effect of S/N due to the low assay range of ELISA doesn't affect the ADA impact assessment on PD.

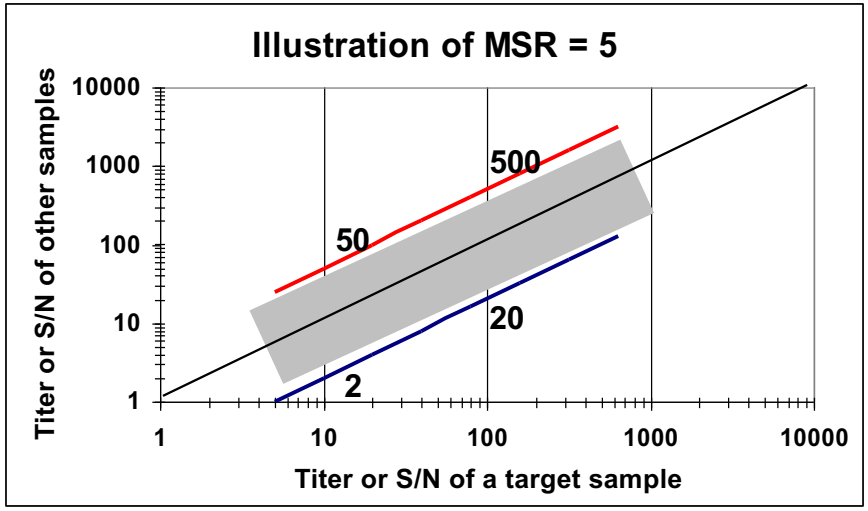
In fact, S/N has a more consistent trend versus PD, with less variability

Review of some background material: MSR & Treatment-boosted ADA

Interpretation of MSR

Minimum Significant Ratio (MSR) *Ref: USP chapter <1106>*

- Useful for defining *Titer or S/N Precision*
- Criteria for Treatment-boosted ADA



Titer of a sample (x-axis) is not significantly different from samples falling in the grey area.

If MSR of titers = 5, and if pre-dose titer = 10, post-dose titer should be > 50 to be treatment-boosted ADA.

Criteria:

MSR < 3 for most assays from our experience and is considered reasonable.

Calculation of Titer MSR

Use the data from the sensitivity experiment (pre-study validation)

- 2-fold serial dilutions of HPC pools (or MPC), >= 3 runs, >= 2 analysts
- Compute titer by interpolating from each dilution curve
- >= 6 titer values (3 runs x 2 analysts)
- Calculate the *SD of log(titer)* results and apply it to the formula below:

$$MSR = 10^{t_{0.05,n\_1} * \sqrt{2} * SD}$$

- Derived from 95% one-sided upper confidence limit of the difference of two results.
- $t_{0.05,df}$ is the two-sided t-distribution threshold for a 5% error rate; n = # of titer results
- Anti-log (10^) of the difference of log(titers) = Ratio of Titers.
- Hence this is the Minimum Significant Ratio of two titer results (T-MSR).

The same formula can be used for calculating MSR of S/N using LPC/HPC data.

In-study PC can also be used for these calculations.

Criteria for identifying treatment-boosted ADA

1. Dilution-dependent criteria (*adapted from clinical serology*)
   - If the titers are determined via 2-fold serial dilutions, a 4-fold difference between pre-dose vs. post-dose titers is suggested as a criterion for treatment-boosted ADA. For 3-fold serial dilution, a 9-fold difference is suggested, etc. This is apparently common in other applications.
   - *Ignores assay & biological variability & doesn't control error rates. This may lead to under-reporting ADA incidence. For e.g., even if titers are diluted 2-fold, differences of 2 to 3-fold between pre-dose vs. post-dose titers may be statistically significant for many assays.*
2. MSR:
   - MSR can be used as a criterion for defining treatment-boosted ADA
   - *(Ref: USP chapter <1106.1>).*

Back to S/N & Titer

Identification of treatment-boosted ADA

Table III Determination of Treatment Boosting for Representative Pre-existing Antibody-Positive Subjects (Assay A15)

Subject	Visit (day)	ADA result	Titer	Boosted by titer (4-fold)	*S/N*	Boosted by *S/N* (4-fold)	Boosted by *S/N* (*MSR*)	*S/N* − 1	Boosted by *S/N* − 1 (4-fold)	Boosted by *S/N* − 1 (*MSR*)
1	1	Positive	100	-	1.18	-	-	0.18	-	-
	15	Positive	3200	Yes	24.88	Yes	Yes	23.88	Yes	Yes
	29	Positive	800	Yes	12.46	Yes	Yes	11.46	Yes	Yes
	85	Positive	12,800	Yes	254.85	Yes	Yes	253.85	Yes	Yes
	169	Positive	51,200	Yes	543.24	Yes	Yes	542.24	Yes	Yes
16	1	Positive	100	-	1.17	-	-	0.17	-	-
	29	Positive	100	**No**	1.84	**No**	Yes	0.84	Yes	Yes
	85	Positive	25,600	Yes	474.31	Yes	Yes	473.31	Yes	Yes
	169	Positive	1,638,400	Yes	1092.38	Yes	Yes	1091.38	Yes	Yes
17	1	Positive	100	-	1.18	-	-	0.18	-	-
	15	Positive	100	**No**	5.86	Yes	Yes	4.86	Yes	Yes
	169	Positive	204,800	Yes	1534.61	Yes	Yes	1533.61	Yes	Yes
46	1	Positive	100	-	3.49	-	-	2.49	-	-
	29	Positive	100	No	2.44	No	No	1.44	No	No
	85	Positive	100	No	1.65	No	No	0.65	No	No
	169	Positive	100	No	1.39	No	No	0.39	No	No

MSR = 1.16 for *S/N* and *MSR* = 1.19 for *S/N* − 1. *MSR* was calculated using in-study precision *S/N* or *S/N* − 1 data for low and high positive controls as validation inter-assay precision data covering a wide range of ADA concentrations was not available

MSR minimum significant ratio

Bold values indicate scenarios where boosting conclusions differed depending on the approach used

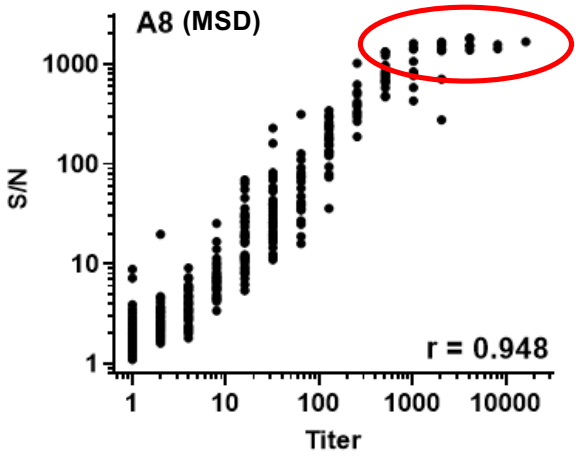
The S/N approach is more sensitive for identifying treatment-boosted ADA than the Titer approach.

Table-III, Starcevic Manning et al., AAPS J. 2022 Jul 12;24(4):81

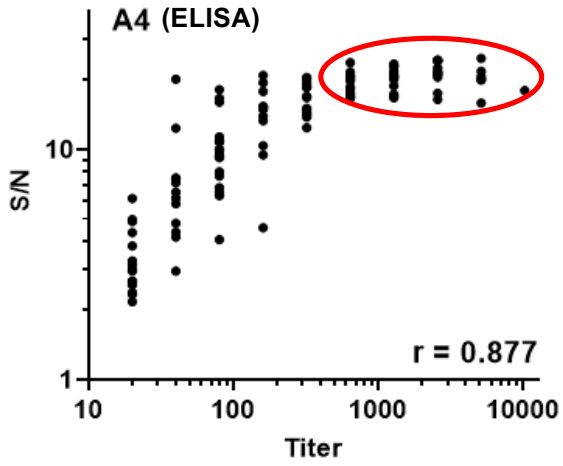
Factors impacting S/N and Titer measurements

Assay range (S/N plateau)

- Most MSD assays had adequate assay range without S/N plateau. The few that had S/N plateau affected < 10% of the tested study samples, and occurred at very high S/N.



- ELISA assay range didn't seem adequate, S/N plateau was quite pronounced.
  - Did not affect the clinical impact assessment of S/N on PK/PD. In fact, the S/N association with PD was more consistent with less variability.
  - Needs to be evaluated carefully. Titer may be a better option in some cases.



Factors impacting S/N and Titer measurements

Precision

- The precision (%CV) of S/N was considerably better compared to Titer
  - %CV ranged from 9% to 24% for S/N, and 27% to 64% for Titer.
- Potential reasons for higher variability of Titer results
  - Lower resolution for low ADA samples
  - Use of endpoint titer method instead of interpolation
  - Extensive serial dilution

Low Titer plateau (high S/N and low Titer)

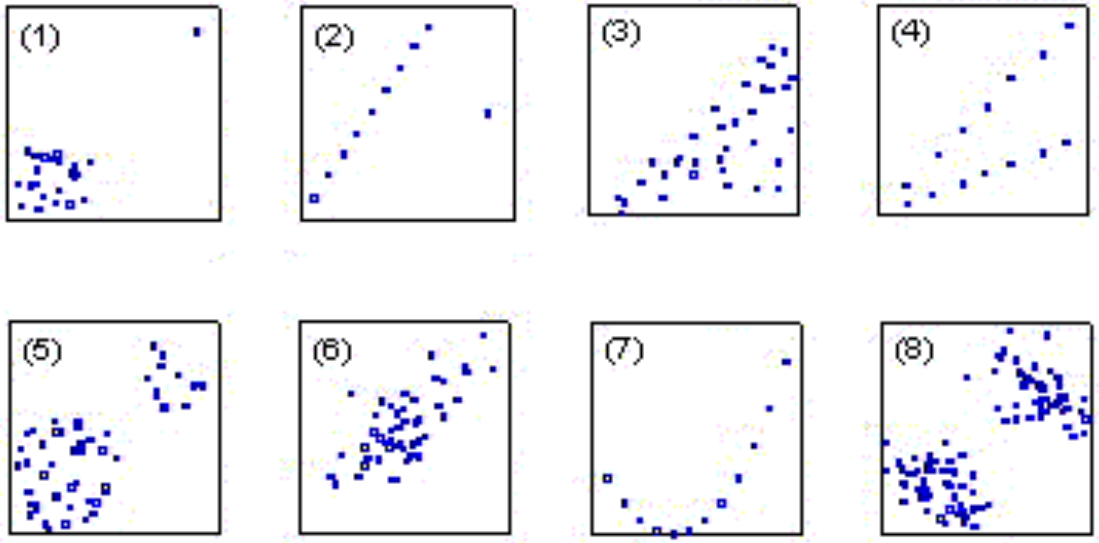
- Possibly due to low affinity/avidity. All were pre-existing positive or placebo.
- High S/N may be real, and may be clinically relevant (lower PK)

Drug and Target interference

- Suitable data were not available for the assays. Should be carefully evaluated during validation.

Always look at the graph(s), not just the numbers!

Correlation (r) = 70% in all these datasets



Graphical Methods for Data Analysis
by Chambers et al.

Graph #6 is the one we typically assume when a correlation of 70% is reported.

The other 7 scenarios here with the same 70% correlation are confounded by different anomalies in the data; e.g., nonlinearly, two subgroups, outliers, etc.

Summary

	S/N	Titer
Pros	1. Simple, efficient, fast 2. More precise 3. Improved differentiation of low-level ADA 4. More robust to low affinity/avidity responses 5. Less reagent use and sample volume	1. Terminology better understood 2. No assay saturation issues 3. Potentially better drug tolerance
Cons	1. Assay range limitations (especially ELISA) 2. Potentially less drug tolerant	1. Inferior precision due to extensive sample manipulation and poor resolution 2. Delayed data availability 3. Increased sample volume, reagent use, cost **4. Historically not validated with the same rigor as screening assays**

S/N was strongly correlated with Titer in most studies. Lower correlations were usually due to higher imprecision of Titer, limited assay range, or the plateau of titer or S/N.

S/N approach can be justified during validation by assessing the factors impacting S/N and Titer (assay range, precision, drug/target interference, low affinity/avidity, etc.).