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To the editor:

Utility in prognostic value added by molecular profiles for diffuse large B-cell lymphoma

We read with interest the report from Hong et al[1] on the incremental prognostic value of gene expression signatures in diffuse large B-cell lymphoma (DLBCL). We were surprised by the conclusions that these "signatures are inferior to clinical factors and provide little added value in risk assessment" when considering a 2-gene score (TGS) and a 6-gene score described by us[2-4] and others.[5] The authors' claim that "all studies assess predictive significance based on *P* value from multivariable Cox regression" was also surprising, because dedicated parts of prior studies specifically addressed added prognostic value. We reported robust risk reclassification by integration of molecular indices (TGS) with clinical factors within the international prognostic index (IPI) (TGS-IPI),[2] and both our study and the study of Lenz et al[5] demonstrated splitting of IPI strata using molecular signatures.

We favored risk reclassification as a measure of added value from molecular indices over more complex statistical tests for several reasons (pie charts in figure 4B of Alizadeh et al[2]). First, even statistically significant biomarkers may yield minimal improvement in the area under the curve.[6,7] Therefore, many statisticians consider C-statistics unsuitable for assessing improvement in prediction, preferring the use of measures such as net reclassification improvement and integrated discrimination improvement (IDI).[7-10] Second, C-statistics and IDI lack an intuitive interpretation for clinicians. However, most surprisingly, we came to the opposite conclusion as the authors in considering these indices in 3 cohorts of DLBCL patients (n = 561), including an important validation cohort that they did not consider. We provide these new data here (Figure 1A), having originally opted not to present them for space considerations.

Although Hong et al do not provide detailed methods to fully address this, several aspects of the analysis may have led to the discrepancy in their conclusions.

1. There is an error in the LIM domain only 2 (rhombotin-like 1) (LMO2) coefficient (0.032 instead of 0.32) for calculating the TGS.

2. The model parameters we reported weight gene expression values measured by quantitative real-time reverse-transcription polymerase chain reaction, with array data needing appropriate rescaling. The TGS values obtained by the authors are thus incorrect, because rescaling is equivalent to changing the relative weightings of the genes in the model, similar to how an equation considering "age" would differ depending on whether it was measured in days or years.

3. We used a custom gene-level chip definition to renormalize probe-level Affymetrix array data from Lenz et al,[5] providing more accurate gene expression quantification (Figure 1B-C).

4. The publicly available normalized expression data used by the authors are already $\log_2$ transformed. Doing this again as suggested in the methods would result in data distortion (Figure 1D).

A

Discrimination Measure, (Significance Estimate)	DLBCL1 Dataset[5] [RCHOP, n=233]	DLBCL2 Dataset[5] [CHOP, n=181]	DLBCL4 Dataset[2] [RCHOP, n=147]
IDI, (p-value)	0.12 (0.0002)	0.07 (0.006)	0.09 (0.0053)
NRI infinite strata, (p-value)	0.64 (0.0003)	0.48 (0.0029)	0.58 (0.003)
NRI predefined strata, (p-value)	0.30 (<0.0001)	0.14 (0.0003)	0.09 (0.0052)

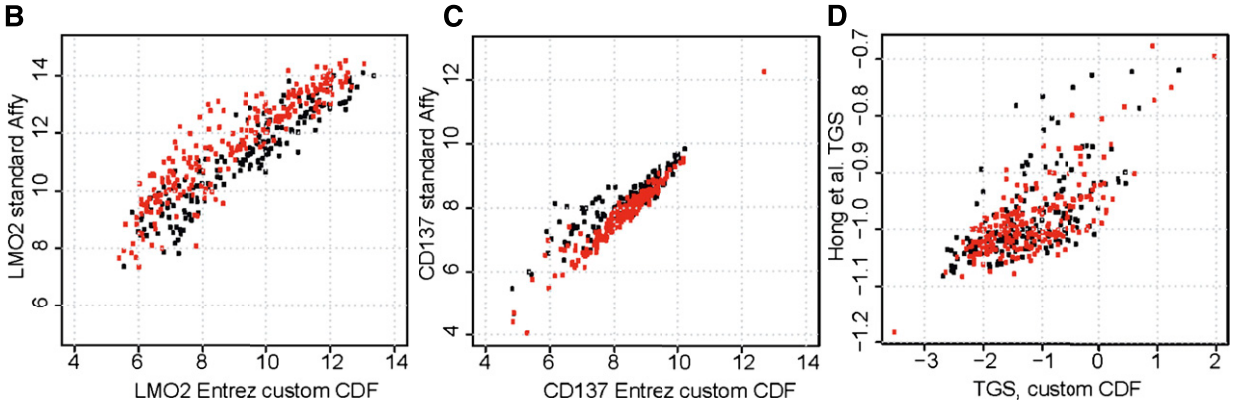

Figure 1. Gene expression signatures add to risk assessment in DLBCL. (A) Estimates of prognostic value added by consideration of expression of two genes (TGS) to the five components of the IPI for predicting risk of death with DLBCL reflect significant improvement in a combined risk model (TGS-IPI) than with clinical indices alone (IPI). NRI, net reclassification improvement.[9] NRI was considered both with infinite strata and with 3 predefined risk strata considering 0% to 10%, 11% to 30%, and >30% risk of death within the follow-up interval. Use of alternate microarray probe set summarization methods to estimate expression levels of (B) *LMO2* and (C) *TNFRSF9* and double logarithm transformation result in significant distortion of the (D) TGS, whether in patients treated with CHOP (black) or RCHOP (red).

5. The authors state that gene expression scores do not add to "clinical factors," but it is not stated whether they considered the 4 original IPI risk groups or its individual components, which yield up to 6 strata. If the latter, then the authors have refit (over-fit) the components of IPI to the test cohort and instead should have compared IPI to TGS-IPI, a composite model that we defined and tested in independent cohorts receiving the R-CHOP regimen (combining Rituximab with Cyclophosphamide, Hydroxydaunorubicin, Oncovin [vincristine], and Prednisone) (Figure 1A).

Importantly, the authors only assess TGS (derived in patients receiving rituximab) in an older cohort of patients not receiving rituximab. This is of little direct clinical relevance because RCHOP is the current therapeutic standard for DLBCL. They also suggest that gene signatures may provide additional information only in intermediate-risk patients. We argue that this is exactly the population where more refined prognostic indices are most valuable, because these are the patients for whom it is most difficult to make therapeutic decisions based on the current care standards.

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To the editor:

Does increased red blood cell deformability raise the risk for osteonecrosis in sickle cell anemia?

The pathogenesis of osteonecrosis in sickle cell anemia (SCA) remains unknown. Blood hyperviscosity has been suggested as a factor involved in the genesis of osteonecrosis,[1] but has not been studied until now. We hypothesized that abnormal hemorheology could play a role in this complication. Hematologic and hemorheologic parameters were assessed in SCA patients with (OST+; n = 30) or without (OST−; n = 67) osteonecrosis. Osteonecrosis was diagnosed as previously described.[2] The study was conducted according to the Declaration of Helsinki guidelines and was approved by the Regional Ethics Committee. The results are reported in Table 1. OST+ patients were older than OST− patients ($P < .05$) and more had a history of vaso-occlusive crises (VOC) within the previous year ($P < .05$) and a higher frequency of α-thalassemia ($P < .05$), confirming previous studies.[3-5] Although the OST+ group exhibited higher hemoglobin (Hb) and hematocrit and a lower hemolytic component than the OST− group ($P < .01$), blood viscosity was not significantly different between the 2 groups ($P < .20$). In contrast, red blood cell (RBC) deformability ($P < .001$) and aggregation ($P < .05$) were increased in the OST+ group. The hydroxyurea (HU) treatment frequency was not significantly different between the 2 groups ($P < .20$). As HU is known to modulate RBC deformability,[6] we analyzed the data as a function of HU therapy independently of osteonecrosis and found that HU-treated patients had lower blood viscosity and greater RBC deformability (data not shown). Excluding HU-treated patients from the cohort did not change the results (Table 1).

A binary (OST−/OST+) multivariate logistic model was used to identify factors associated with osteonecrosis in SCA patients and included age, Hb, RBC aggregation and deformability, hemolytic component, α-thalassemia status, and previous history of VOC as covariates. The overall model was significant

Table 1. General characteristics and hematologic and hemorheologic parameters in patients with (OST+) and without (OST−) osteonecrosis

	With patients undergoing HU treatment		Without patients undergoing HU treatment	
	OST− (n = 67)	OST+ (n = 30)	OST− (n = 57)	OST+ (n = 22)
Age (y)	32.5 ± 12.2	39.3 ± 13.1*	32.0 ± 12.4	38.8 ± 11.9*
Gender (male/female)	32/35	11/19	27/30	9/13
HU (%)	15.9	27.6	—	—
α-Thalassemia (%)	37.3	56.7*	40.4	59.1
Positive history of VOC (%)	9.0	26.7*	8.8	27.3*
HbF (%)	7.9 ± 5.7	9.6 ± 6.2	7.5 ± 5.6	9.1 ± 6.1
WBC (10^9/L)	9.5 ± 2.0	8.7 ± 2.1	10.0 ± 2.7	9.0 ± 1.7
RBC (10^{12}/L)	2.8 ± 0.6	2.9 ± 0.5	2.8 ± 0.6	3.1 ± 0.4
PLT (10^9/L)	404 ± 126	381 ± 136	414 ± 125	373 ± 144
MCV (fL)	83.5 ± 9.8	86.6 ± 10.1	81.4 ± 8.2	83.5 ± 7.4
MCHC (g/dL)	35.9 ± 1.1	35.6 ± 1.2	35.8 ± 1.1	35.5 ± 1.3
Hb (g/dL)	8.2 ± 1.3	9.0 ± 1.1**	8.1 ± 1.2	9.1 ± 1.1***
Hct (%)	22.9 ± 3.7	25.2 ± 3.0**	22.7 ± 3.4	25.6 ± 3.1***
RET (%)	8.5 ± 3.3	7.7 ± 2.7	8.6 ± 3.3	7.7 ± 2.3
BIL (μmol/L)	61.9 ± 44.1	52.6 ± 37.4	62.8 ± 46.1	54.7 ± 43.0
AST (IU/L)	39.4 ± 14.8	37.0 ± 10.1	39.8 ± 14.3	37.1 ± 11.2
LDH (IU/L)	522 ± 166	433 ± 96**	537 ± 161	442 ± 100**
Hemolytic component (relative unit)	0.16 ± 1.10	−0.35 ± 0.61**	0.23 ± 1.08	−0.33 ± 0.61**
ηb (mPa/s)	7.64 ± 1.79	8.24 ± 2.01	7.80 ± 1.75	8.40 ± 2.16
RBC deformability at 3 Pa (a.u. × 100)	15 ± 6	20 ± 5***	15 ± 5	19 ± 5***
RBC aggregation (%)	52 ± 9	57 ± 8*	52 ± 10	55 ± 7
RBC disaggregation threshold (s^{-1})	306 ± 148	262 ± 108	309 ± 152	265 ± 116

Values are means ± SD. All patients were at steady state at the time of the study, ie, no blood transfusions in the previous 3 months and absence of acute episodes at least two months before inclusion into the study. Measurements of 4 hemolytic markers (BIL, bilirubin; LDH, lactate dehydrogenase; AST, aspartate aminotransferase; RET, reticulocytes) were performed using standard methods, and a principal component analysis was used to derive a hemolytic component value from these markers.[9] This standard statistical data reduction approach uses conventional clinical measurements to explain the maximum-shared variance among these indirect measures of hemolysis. The hemolytic component has recently been demonstrated to reflect intravascular hemolysis,[9] had a mean of 0 (standard deviation = 1.0), and predicted 49.2% of the variation among all 4 measured variables (eigenvalue = 1.97). Blood viscosity, RBC deformability, and aggregation properties were determined as previously described.[10] Polymerase chain reaction (Gap-PCR) was used to detect the 6 common α-thalassemia deletions, including $-\alpha^{3.7}$ and $-\alpha^{4.2}$ alleles, and triplication defects of the α-globin genes.

HbF, fetal Hb; Hct, hematocrit; MCHC, mean corpuscular hemoglobin concentration; MCV, mean cell volume; PLT, platelets; VOC, vaso-occlusive crisis; WBC, white blood cell; ηb, blood viscosity.

Significant difference between the 2 groups: *$P < .05$; **$P < .01$; ***$P < .001$.