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Original Research

A Network Phenotyping Strategy approach in a Turkish HCC Dataset and Comparison of Patients Selected for Transplant and those who were not

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Abstract

Objectives: A Network Phenotyping Strategy (NPS) was recently created to stage hepatocellular carcinoma (HCC) from an Italian dataset into 25 discrete phenotypes sT, s=1→τ_1, …, s=25→τ_{25} ordered (τ_1<τ_2,…,<τ_{25}) according to the dynamics of the HCC progression from its onset.

Methods: To use NPS methodology on an ethnically different, Turkish HCC cohort that had, in addition, been stratified according to patients selected for liver transplantation or not.

Results: The Turkish patients had only a smaller subset of 16 out of the 25 HCC phenotypes of the Italian patients. HCC progression through phenotypes, which are exclusive to the Italian population, is a dominantly tumor biology-driven process, occurring within a constant extent of liver microenvironment damage. In contrast, in phenotypes shared by the minority of Italian patients and the majority of Turkish patients, the HCC progresses by a more complex disease burden generating mechanism, consisting of simultaneous tumor biology-driven damage, accompanied by advancing liver microenvironmental impairment.

NPS objective stratification of a "real world clinical practice" patient cohort into subpopulations with identical HCC clinical phases enabled the simulation of clinician-dependent selection for liver transplantation or not in this cohort, using specific baseline variables. A clearer understanding of HCC biology has allowed us to identify differing biological phenotypes. The predominant 12T phenotype was examined in detail and combined with surgical knowledge obtained retrospectively as to the difference between transplanted and non-transplanted patients to then derive models that may be useful for biology-dependent surgical decision support in future patients.

Conclusion: Only a subset of all HCC phenotypes appeared in the Turkish cohort and may be explained by the non-screened patients having HCC as a secondary problem, and is primarily driven by the liver microenvironment, in contrast to Italy, where HCC develops (predominantly) in healthier livers.

Keywords: HCC, Network phenotyping strategy, transplant

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A Network Phenotyping Strategy (NPS) is a novel non-empirical clinical analytics methodology, explicitly addressing the essential role of disease dynamics in data-driven stage definition, diagnostic and use in prognosis[1-4] through clinically transparent usage of only baseline multi-dimensional clinical data of a real-world patient cohort. We have shown[1,2] that supplementing the patient and visit coherent values of multiple (K) clinical variables by the topology of a complete network of relationships between those values, encoded quantitatively by special K-partite graphs (see Fig. 1) allowed us to characterize the disease progression stage in terms of the personal time to disease onset at the baseline. This unique innovation of NPS addresses the disadvantage of the above cited methods, which do not explicitly include mechanisms for normalizing the patient data change rates according to the biological stage of the disease at baseline.

We recently used a Network Phenotyping Strategy to identify the stages of the disease in patients with hepatocellular carcinoma (HCC) as progression-ordered phenotypes, using an unstratified large Italian database.[2] The purpose of the current study was to use the same NPS methodology on an ethnically different (Turkish) HCC cohort that had also been stratified according to patients who were selected for liver transplantation or not. These Turkish patients differed in having predominantly Hepatitis B (HBV) as a background and minimal or no alcoholism, unlike the Italian patients. Furthermore, they were predominantly diagnosed without a surveillance program and thus, on average, had more advanced disease. We report that in contrast to the Italian patients, the Turkish patients are diagnosed only within a restricted selection of the full spectrum of progression-ordered HCC phenotypes. Quantitative ordering of the NPS diagnosed HCC stages according to their characteristic τ_s allows for studying the τ_s- dependent trends of characteristic clinical variable values without need for longitudinal data.

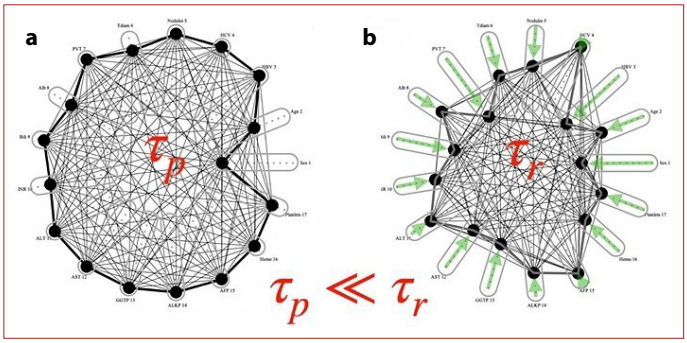

Figure 1. 17-partite graphs $\Gamma_p(\tau_p)$ for two patients with different HCC stages. The 17 ovals represent variables, with internal vertices representing categorical values or sub-intervals of the complete physiological range of respective real-valued variables. Solid circles indicate observed personal baseline values. The network of edges encodes the unique personal clinical context of each variable value. As some specific variable values are typically observed early and other late in disease progression, the topology of the personal relationship network expands or shrinks (see arrows) as the function of baseline time to disease onset. τ_p.

Comparing these trends between the Italian and Turkish patient populations allowed us to hypothesize that observed differences may be due to the HCC in Turkish patients being predominantly driven by the liver microenvironment, in contrast to the more diverse driving factors in European patients. In addition, we used the NPS information about differences between Turkish patients, selected and not selected for liver transplantation at different stages of HCC progression to suggest new insight into the treatment selection and outcomes.

Methods

Patients were characterized by values of 17 standard baseline clinical parameters at initial clinical presentation, with tumor size and number and presence or absence of PVT, based on their initial CAT scan measurements, who also had known survival data. The 17 clinical parameters were chosen based upon baseline routine clinical data that is collected to evaluate any newly-presenting HCC patient and are in 3 groups: A), Demographics that included age [years], gender and HBV/HCV status; B), tumor characteristics, that included maximal diameter (MTD [cm]), tumor uni- or multi-focality, presence/absence of portal vein thrombosis (PVT) and serum α-fetoprotein (AFP [ng/mL]) levels; C), serum liver parameters and blood counts, including levels of albumin [g/dL], total bilirubin [mg/dL], INR, ALT (in relative unit 1/35 [U/L]), AST (in relative unit 1/35 [U/L]), ALKP (in relative unit 1/150 [U/L]), GGT (in relative unit 1/40 [U/L]), Hb [g/dL] and platelets [count/μL].

Ethical Considerations

Database management conformed to legislation on privacy, and this study conforms to the ethical guidelines of the Declaration of Helsinki and approval for this retrospective study on deceased cases and de-identified patients with HCC. This work was approved by the Institutional Ethics Committee (Institutional Review Board Approval No. 2024-6196) for a waiver from obtaining written informed consent for de-identified and mostly deceased patients, in accordance with local guidelines.

Clinical Background of the Analysis

We have two socio-ethnically, institutionally and clinically different populations of patients with the same diagnosed disease (HCC). One (larger, less stratified, surveillance generated) population (ITALICA) provides broad, comprehensive

reference characteristics of HCC progression phenotypes. In the previous study (1), we used the NPS methodology on the surveillance ITALICA cohort, where the broad spectrum of HCC stages is "clinically probed" by individual patient disease statuses. In the other (Turkish), more stratified population, processed by the same NPS algorithm, we expect to see where the real-world "clinical bias" will place these patients in the complete "disease phenotype space" of HCC progression. The goal of this exercise was to obtain better, data-driven personalization of the HCC disease characterization, usable as the decision support information for detailed diagnosis, prognosis or treatment selection. See note 1.

These clinical goals required applying the new, relationship-based NPS method to process the multidimensional (17-variable) characterization of patient clinical profiles. This is because NPS new information, which this method uses to identify the 25 personal HCC progression stages sT, (s=1,…,25) are the networked patient's data relationships, added and quantitatively processed together with conventionally used values of respective clinical variables, collected at the index-visit.

The fact that there are just those 25 progression stages is objectively determined by the NPS processing of the reference ITALICA clinical data relationships in two steps (see ref. 2).

In the first step, the NPS extracts the personal times τ_p from HCC onset from the topologies of observed value relationship networks of every patient.

In the second step, NPS shows that by organizing all patients according to the variability in the stage-dependent presence of early $E_p(\tau_p)$ and late $L_p(\tau_p)$ stage biomarker topologies in those personal networks $\Gamma_p(\tau_p)$, from which τ_p's are computed, the patients emerge naturally grouped into 25 HCC clinically and progression-stage normalized sub-populations, defining the 25 HCC phenotypes, sT. This new NPS-based stage diagnostic of HCC is represented by HCC progression map (see Fig. 2), in which each patient is represented by a τ_p-defined point $[X_p(\tau_p), Y_p(\tau_p)]$, where $X_p(\tau_p)=\log(L_p(\tau_p))$ and $Y_p(\tau_p)=\log(E_p(\tau_p))$. The grouping of all HCC patients into 25 ordered phenotypes emerges naturally from this visualization of this primary result of patient's clinical data processing by NPS.

This new, disease-progression ordered clinical phenotyping information provides 2 practical results:

Firstly, it deconstructs the conventional population averaged data characterizations of the clinical status into the series of 25 objective components, stratified according the progression-ordered sT's.

Secondly, it provides the numerical value of the τ_p-dependent personal clinical burden $CB_p(\tau_p)=Y_p(\tau_p)-X_p(\tau_p)=\log\left(\frac{L_p(\tau_p)}{E_p(\tau_p)}\right)$ for every patient. Consequently, once these are aggregated for all patients into their respective 25 HCC progression stages sT, we newly obtained the objective, quantitative, clinically transparent and characteristic values of progression-specific disease burden, $CB_s=\frac{1}{N_s}\sum_{p_s=1}^{N_s}CB_{p_s}$ for all respective progression stages of HCC.

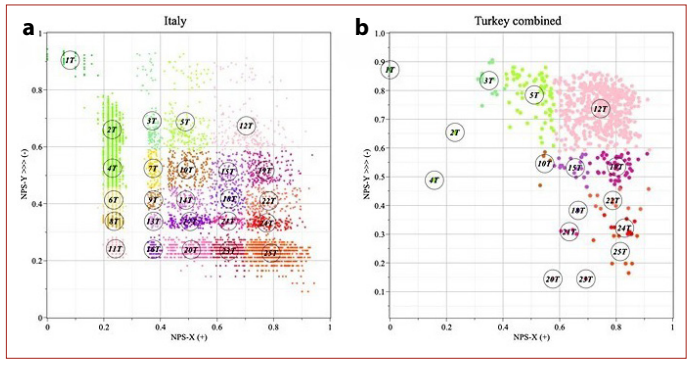

Figure 2. NPS 2-dimensional maps of HCC progression for Italian **(a)** and Turkish **(b)** patients. Each patient is represented as a point. $[X_p(\tau_p), Y_p(\tau_p)]$. X-Y coordinates independently quantify the relative presence of early and late HCC stage networked biomarker topologies in patient's $\Gamma_p(\tau_p)$: 1.0 on the vertical axis and 0.0 on the horizontal axis indicates that we observe 100% of the early stage biomarker network in the patient's graph, while 0.0 on the vertical axis and 1.0 on the horizontal axis indicates that the patient's graph has 100% of the late stage biomarker network. Other values represent personalized combinations of the two prototype topologies and underlying early and late stage biologies sT,(s=1,…,25). The phenotype sub-populations sT,(s=1,…,25) are automatically identified as tight groupings of patients with highly similar clinical profiles: The circles with phenotype labels have centers at the maximum of the exponential distribution of patients in each phenotype region.

Why and how this NPS-Enabled Approach Provides New Clinical Insights

With the exception of higher HBV incidence in Turkish patients and minimal or no alcoholism, other conventional clinical characterization of the 2 populations at the index visit looks very similar, since the frequencies and distributions of the 17 clinical variable values are comparable between the two populations.

The main new clinical information provided by NPS is the de-construction of these single, whole population characteristic values into 25 disease-progression ordered phenotype specific partial averages, computed in sequence by using only data of patients diagnosed with respective HCC progression stages sT. Thus, because we know the characteristic values of $CB_s(\tau_s)$ for each HCC progression stage, we can analyze the clinical HCC progression trends by plotting phenotype-characteristic mean values $\mu_{i,s}=\frac{1}{N_s}\sum_{p_s=1}^{N_s}V$ of respective clinical variables as the function of $CB_s(\tau_s)$.

For supportive computational evaluation of patient's likelihood of being eligible for liver transplant we used multivariate logistic regression regression analysis (JASP software[5]), based upon the baseline data. Starting with all 17 clinical variables, the stepwise, forward, and backward variable selection optimization was performed for patients, identified by NPS analysis in the 12T phenotype (these patients share the same stage of HCC and form 72% of the total population). All optimizations converged to the same best-performing 6-variable model, which includes gender, INR, albumin, hemoglobin, bilirubin, and MTD.

Results

Mean survival of Italian patients is $\mu_{survival}^{Italy}$=1765 days, which is ~1.3 times higher then for Turkish patients $\mu_{survival}^{Turkey}$= 1370 days. Overall mortality (defined as percent of deaths within the 5 year study period) is ~2 times higher in Italy ($\mu_{mortality}^{Italy}$ = 63%) then in Turkish population ($\mu_{mortality}^{Turkey}$=31%). In Turkish subpopulations, stratified by transplant (T) and non-transplant (*nT*) treatment, the $\mu_{survival}^{TT}$=1626 days, $\mu_{survival}^{T,nT}$=710 days, while mortalities are stratified into $\mu_{mortality}^{TT}$=36% and $\mu_{mortality}^{T,nT}$=19%.

In Figure 2 we compared the complete NPS map of all 25 HCC phenotypes *sT*, (s=1,…,25) from processing screening data for 4802 Italian HCC patients, collected by the ITALICA database (Fig. 2a), and the phenotype assignments of the combined Turkish liver non-transplant (nT) and liver transplant (T) HCC patients (N=681 (N_{nT}=191, N_{T}= 490)) (Fig. 2b). In this map, each patient is represented by a point, each phenotype is uniquely colored and the circles with phenotype sT labels have the centers at the maximum of the patient distribution in each HCC progression stage phenotype region (see Fig. 3b and c). Progression stages are ordered according to the increasing time to disease onset τ_s. Therefore, the patients in the 1T region of the map are in the earliest diagnosable HCC stage, while patients in the 25T region of the map are in the latest observed HCC stage. Thus, Turkish patients appear in only 16 of the 25 phenotypes seen in the Italian group, with some of those sparsely populated. Specifically, the Turkish patients are not diagnosed with phenotypes *6T, 7T, 8T, 9T, 11T, 13T, 14T, 16T, 17T*, which, in contrast, are heavily populated by Italian patients.

Figure 3 shows in detail the differences between patient distributions across the HCC phenotypes in detail, determined separately for liver transplant (T) and non-transplant (nT) sub-cohorts in the Turkish HCC population. The red and blue distributions above the NPS-X (horizontal) and NPS-Y (vertical) HCC progression axes of the HCC progression stage map (Fig. 3a) show how stage-characterizing networked clinical relationship profiles of individual

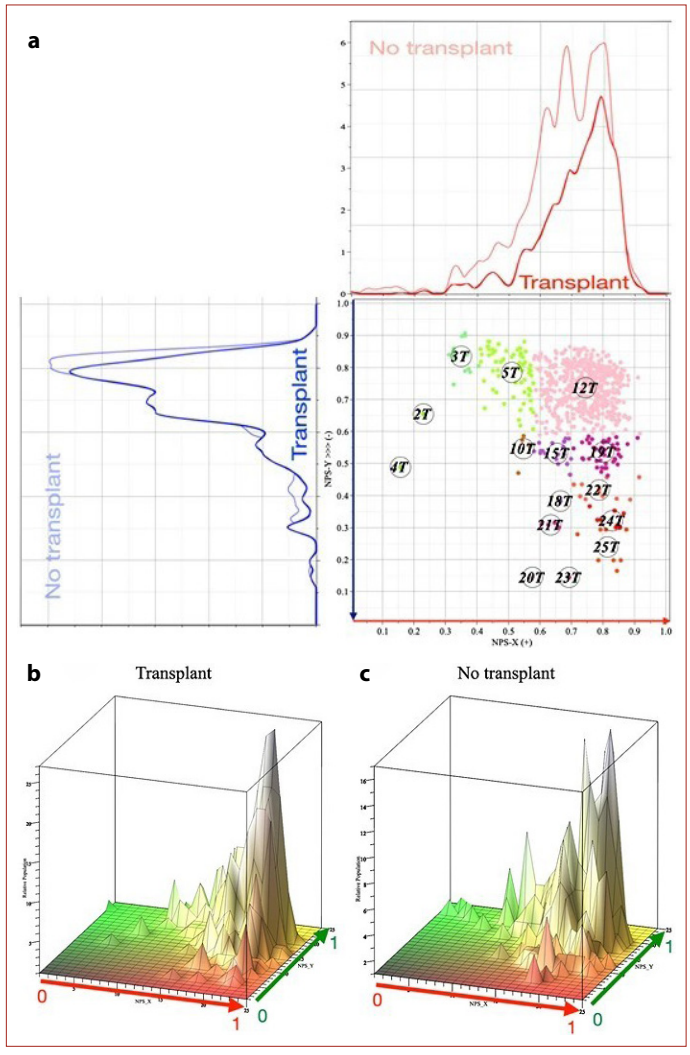

Figure 3. Differences in Turkish patient distribution across HCC phenotypes between transplant and non-transplant subgroups. **(a)** Top and left panels: Projections along the axis capturing the diminishing contribution of earlier stages (blue) and along the late axis capturing the increasing contribution of late stages (red). Light lines: non-transplant, dark lines: transplant. Bottom panels: 3D histograms of patient populations in respective phenotypes for transplant **(b)** and no transplant (**c)** patients.

patients are localized in the two projections of the HCC progression phenotype cases for the T and nT sub-cohorts into characteristics of early HCC stages (Y-axis, blue distributions) and those of later HCC stages (X-axis, red distributions). In the early HCC stages, the levels of NPS early-stage biomarkers in patients's Γ_p (τ_p) are distributed comparably in both treatment-defined groups. In contrast, in the later HCC stages, the late clinical profile biomarker levels in the (smaller) nT patient sub-cohort Γ_p (τ_p)'s span a broader interval than those for the T patients. This corresponds to application of the standard liver transplant selection criteria for these patients. Figure 3b-c show the complete 3D distributions of patients in the LT and nT sub-cohorts. There is

a clear separation of the sub-distributions (peaks) in these 3D distribution plots, delineating the patients with respective HCC stage phenotypes, which, consequently, quantifies the high level of personal clinical similarity of biologies within every patient phenotype sub-population.

We then outlined the NPS-extended experimental characterization of clinical differences between the Italian and Turkish HCC patients, which leads to the elective location of the Turkish cohort in the complete HCC progression phenotype map. For that comparison, we used the transformation of the primary 2-dimensional NPS information about each patient, defined by the coordinate pair [$X_p(\tau_p)$,$Y_p(\tau_p)$] into one-dimensional, HCC progression phenotype specific clinical burden parameter $CB_p(\tau_p)=Y_p(\tau_p)-X_p(\tau_p)=log\left(\frac{L_p(\tau_p)}{E_p(\tau_p)}\right)$. This transformation permitted a study of the trends of 16 phenotype-characteristic mean values $\mu_{i,s}$ of respective clinical variables against the corresponding 16 phenotype-characteristic values of time to disease onset τ_s dependent clinical burdens $CB_s(\tau_s)$.

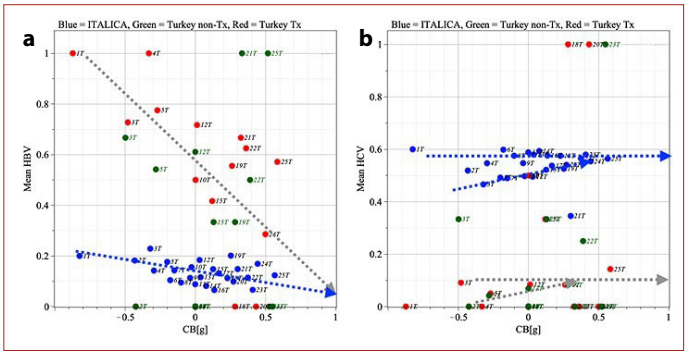

Figure 4. Dependence of phenotype sT-characteristic incidences of HBV **(a)** and HCV **(b)** (vertical axes) on the phenotype characteristic, τ_s-dependent clinical burden $CB_s(\tau_s)$ (horizontal axes) - see Methods for $CB_s(\tau_s)$ definition. Blue points: data for Italian patients, green points: data for Turkish non-transplant patients, red points: data for Turkish transplant patients.

In the Turkish HCC population, relatively more males were found in T patients, and with few exceptions, nT patients in all sT phenotypes were slightly older than T patients. On average, Turkey patients were 1.3 younger than Italy patients (μ_{age}^{Italy}=67.5, μ_{age}^{Turkey}=53.5, for Turkey $\mu_{age}^{T,T}$=53.7, $\mu_{age}^{T,nT}$= 52.8).

In the following figures, the data for Italian patients are shown by blue points, and comparisons between T and nT Turkish treatment-based sub-groups for stage-characteristic values of respective clinical variables in their HCC patients is shown by green (nT) and red (LT) points, representing the paired values $[CB_s(\tau_s), \mu_{i,s}^{T,X}]$.

In Figure 4a the higher incidence of HBV cases in the Turkish compared to the Italian patients is shown by the systematic vertical offset of the two trends (gray arrow for Turkey, blue arrow for Italy). This difference is accompanied by a lower overall incidence of HCV in the Turkish cohort, resulting in a systematic vertical offset of the two trends in Figure 4b.

Processing the relationship-networked patient clinical data by NPS resulted in novel, more clinically informative and disease-progression based insight into this conventional characterization of overall hepatitis incidence by just the differences in two means. Figure 4 shows, that within the respective HCC progression-ordered phenotype patient subgroups, the incidence of hepatitis exhibits clear trends in both cohorts. HBV incidence in HCC patients is maximal at the early HCC stage patients and decreases proportionally with the HCC progression, defined by the corresponding $CB_s(\tau_s)$ (arrows in Fig. 4a). In contrast, the HCV incidence increases moderately in both cohorts as the HCC progresses to late stages (arrows in Fig. 4b).

The trends of progression of tumor characteristics (mean MTD and number of tumor nodules as the function of corresponding $CB_s(\tau_s)$) are increased in both populations with increasing disease stage (Fig. 5a, Fig. 5b), as expected. They

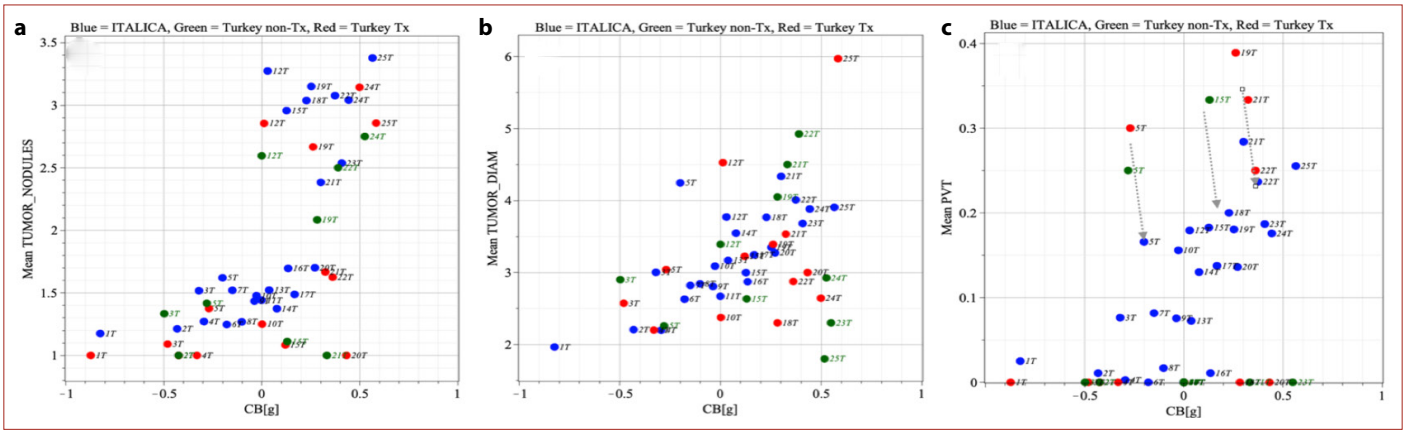

Figure 5. Dependence of phenotype sT-characteristic values of tumor nodule numbers **(a)**, maximal tumor diameters **(b)**, and PVT incidences (vertical axes) on the phenotype characteristic τ_s-dependent clinical burden $CB_s(\tau_s)$ (horizontal axes). Blue points: data for Italian patients, green points: data for Turkish non-transplant patients, red points: data for Turkish transplant patients.

are on average parallel in both populations, just with expected moderately smaller tumors in the late HCC stages in Turkish patients, who were selected for liver transplant (Fig. 5b).

In the previous (Italy-based) report (2), we identified 5 groups of 25 HCC progression phenotypes sT, each group with a unique, constant characteristic value of PVT incidence. We found in the Turkish patients (Fig. 5c) a qualitatively similar, PVT-incidence characteristic partitioning of those sT phenotypes, just with quantitatively higher PVT incidence in the top 2 PVT groups in the Turkish than for the Italian patients in the equivalent phenotypes (arrows in Fig. 5c).

Figure 6 shows comparisons of HCC progression for the remaining parallel trends in both groups. Phenotype characteristic total bilirubin and INR values increased with HCC progression (Fig. 6a, 6b), consistent with increasing hepatocyte damage. As the clinical burden increased, the albumin decreased, also consistent with parenchymal destruction by growing tumor. The decreases in both albumin (Fig. 6d) and hemoglobin (Fig. 6e) are consistent with the reported nutritional deficiency and tumor-associated inflammation for growing tumors, as in the Glasgow index.[6] The AFP trended up with increased disease burden in both the Turkish and Italian cohorts (Fig. 6c), but with a large scatter, and with higher absolute values in the Italian than the Turkish cohort. AFP and albumin appear to have an inverse trend (Figs. 6c and 6d), as has been previously shown,[7] as they are in the same family of proteins, with one influencing the other.[8] In this case, standard statistical analysis using absolute values and the NPS approach using trends, showed a very similar result and clinical conclusion in terms of trends. Platelets trended down (Fig. 6f) with increase in tumor burden, likely associated with increasing cirrhosis. For AFP, hemoglobin and albumin, the phenotype characteristic values decrease with HCC progression. The main differences between the Turkish and Italian patients are in relative quantitative shifts of these synchronous trends.

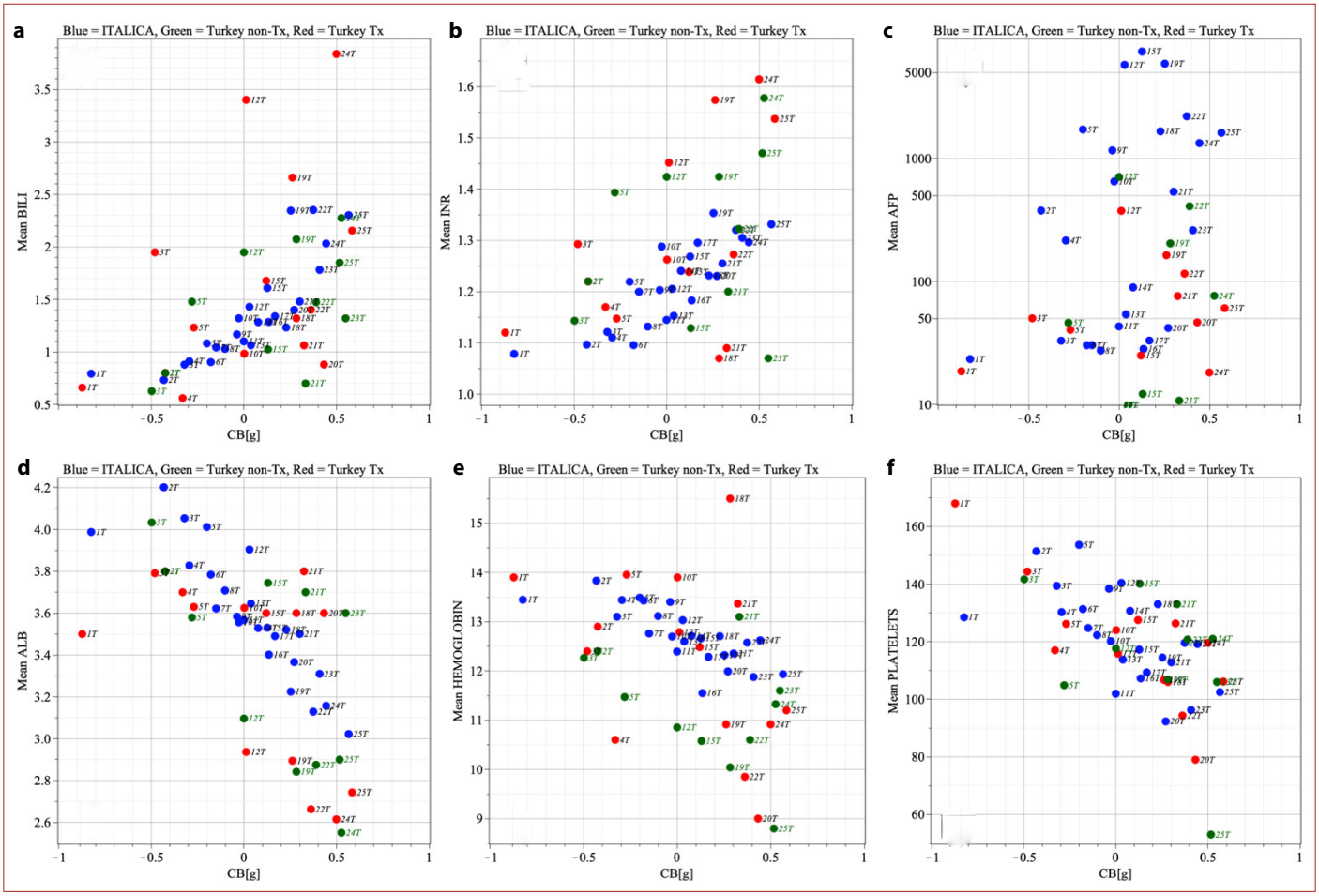

Figure 6. Dependence of phenotype sT-characteristic values of bilirubin **(a)**, INR **(b)**, AFP **(c)**, albumin **(d)**, hemoglobin **(e)** and platelets **(f)** (vertical axes) on the phenotype characteristic τ_s-dependent clinical burden CB_s (τ_s) (horizontal axes). Blue points: data for Italian patients, green points: data for Turkish non-transplant patients, red points: data for Turkish transplant patients.

The ALT, AST, ALKP and GGT levels (Fig. 7a-d) are all also systematically higher in the Turkish patients. In addition, in the Turkish patients the phenotype-characteristic ALT, AST and GGT level dependence on HCC progression is opposite (clearly decreasing trend) compared to the Italian patients (moderately increasing trend), and quite similar to the HBV trends of Fig 4. The CB_s (τ_s)-dependent trends of these variables in both cohorts converge to similar characteristic values at CB_s (τ_s)=1. Note also, that in the Italian cohort, there are patients diagnosed in the sT phenotypes, dominantly populated in Turkish patients (1T, 5T, 12T etc.), with the phenotype characteristic levels of these variables and CB_s (τ_s)-dependent trends align ing with the Turkish patients.

A comparison of the outcome characteristics between the Turkish nT and T patients was then made (Fig. 8). Transplant clearly improved survival duration, as expected. New information by NPS is provided by the possibility for studying the trends in survival prolongation as a function of the characteristic HCC progression stage time to

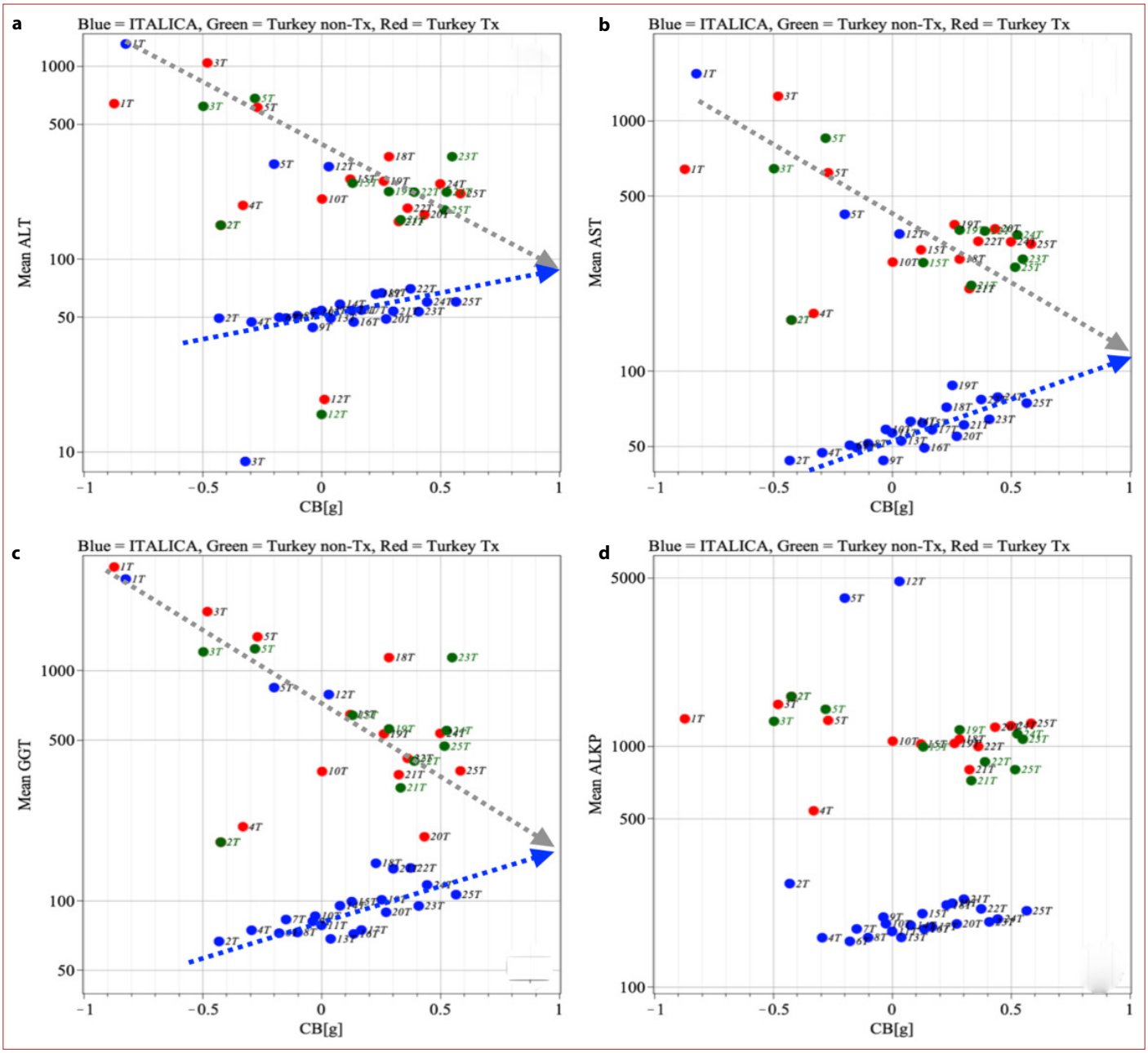

Figure 7. Dependence of phenotype sT-characteristic values of ALT **(a)**, AST **(b)**, GGT **(c)**, and ALKP **(d)** (vertical axes) on the phenotype characteristic τ_s-dependent clinical burden CB_s (τ_s) (horizontal axes). Blue points: data for Italian patients, green points: data for Turkish non-transplant patients, red points: data for Turkish transplant patients.

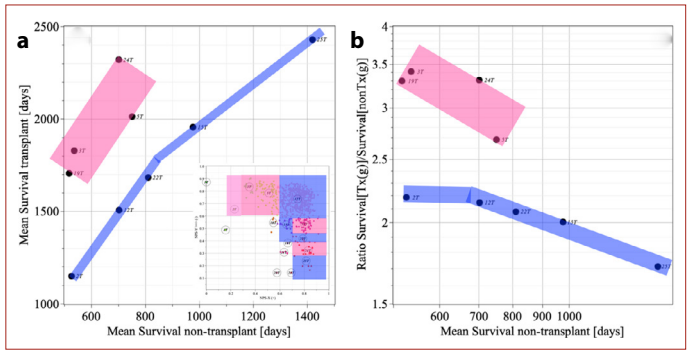

Figure 8. (a) Relationship between the mean survivals of patients in the corresponding HCC phenotypes sT for non-transplant (values on horizontal axis) and transplant (values on vertical axis) groups. Magenta and blue boxes outline the two phenotype subgroups with different survival improvements. The survival improvements in respective groups are also shown by the corresponding color in the HCC progression map (insert). **(b)** Ratios of mean survivals for transplant to mean survivals for no transplant patients in respective sT phenotypes for respective mean survivals in no transplant group. The colors of the boxes are for the same sT groups as in **(a)**.

disease onset τ_s. This shows that there are 2 treatment groups, shown by magenta and blue boxes. In the magenta box, there are larger differences in survival between T and nT patients (e.g. for 5T patients in the magenta box, patients treated with transplant had a mean 2000 day survival, but without transplant they had an approximately 760 day survival, an approximately 2.7 fold survival difference, as shown for 5T in Figure 8b, magenta box. For the 12T phenotype (middle of the trend in the blue box, which is the most populated phenotype, T patients had a 1500 day mean survival, whereas nT patients in the same 12T phenotype had only a 700 day mean survival, or a 2.1 fold survival difference, as shown in 12T in the blue box of Figure 8b. Qualitatively, there are 2 sub-populations with different prolongation ratios (Fig. 8b). In one sub-population, the T patients in the "magenta" progression phenotypes (identified by magenta color in Fig. 8a insert) had a 2.7 to 3.5 times longer typical length of survival than clinically identical nT patients. In the second sub-population in contrast, the T patients in the "blue" progression phenotypes (Fig. 8a insert) had only a 1.7 to 2.2 longer typical length of survival than the clinically identical nT patients. Thus, NPS approach shows the relative treatment advantages for patients, newly diagnosable in multiple phenotypes of HCC that are not differentiable in current clinical practice. The NPS phenotypes treated by T or nT have clinically relevantly different survivals, depending on whether they are in the blue or magenta groups of HCC phenotypes (HCC progression map, see Fig. 8a, insert). In addition, there are NPS-discovered trends in the relative outcomes in both magenta and blue identified phenotype groups, whose patients will benefit more in survival after T compared to nT, depending on phenotype, into which their HCC disease progressed at baseline (Fig. 8).

Logistic Regression Model for Transplant Versus Non-Transplant

Having used NPS to identify the 12T phenotype as containing the majority of the Turkish transplant and non-transplant patients, we then performed standard statistical analysis to identify 6 significant parameters between the 2 Turkish treatment (T and nT) sub-cohorts (Table 1). Table 1 shows the best-performing logical regression model for 12T patients. This recommendation follows from our previous observation (2) that developing the conventional prognostic models for the patients, assigned to clinically identical disease stages by NPS improved the quality, performance and clinical relevance of these models. This improvement follows from removal of randomness in the disease stages in the training "real world" clinical data, which any conventional approach suffers from.

$$P = \frac{1}{(1+e^{-(-4.150-0.724\times gender+0.667\times INR+0.565\times ALB-0.518\times Hb-0.468\times BILI-0.113\times MTD)})}$$

We show above the explicit form of the optimal logistic regression equation for calculating the prognostic value P for transplant treatment eligibility for the most populated Turkish phenotype 12T, using the baseline visit values and weights of 6 best predicting variables, shown in Table 1: If the prognostic value, calculated by substituting the 6 respective personal baseline variable values into the above equation (male code = 0, female code = 1) is P≥0.55, then the patient with diagnosed 12T stage of HCC is categorized as likely to be found eligible for transplant in the Inonu liver transplant institute.

Table 1. Logistic linear regression model for assessing the probability to be selected for liver transplant (threshold = 0.55).

	Intercept	Gender (Female=1)	INR	Alb	Hb	Bilirubin	MTD
Coefficient	-4.150	0.724	-0.667	-0.565	0.518	0.468	0.113
Odds ratio	0.016	2.1	$(2.0)^{-1}$	$(1.8)^{-1}$	1.7	1.6	1.1
p	<0.001	0.006	0.009	0.003	<0.001	<0.001	0.003
Error	1.15	0.4	0.4	0.2	0.06	0.1	0.04

Discussion

NPS provides new information from otherwise "standard" data by adding the conventionally neglected inter-relationship information between the co-observed personal values of 17 clinical variables to the normally used information about only the personal values of respective clinical variables. This leads to clinical analytics approaches standardly using only the population-characterizing mean values (say mean HBV incidence in Italian vs. Turkish cohorts and similarly for bilirubin and other parameters). In contrast, and newly-described here, NPS deconstructs these single value pairs into multiple mean values in respective HCC progression phenotype, sT's, which importantly and again newly, are objectively ordered according to increasing τ_p. Thus, for clinical interpretation, we can improve the conventional frequentist's statistics by discussing actual trends of characteristic clinical variable values as a function of the objectively determined, τ_p-dependent HCC progression stage (Figs. 4-8). These trends are a generalization of the conventional, value-based clinical comparisons (meaning that one can see those conventional overall averages distributed in time into the trends), and are generated by NPS without longitudinal data. This approach has the potential for using the new information in the treatment/no treatment context.

The NPS method is clinically unique because it analytically determines the personal times to disease onset τ_p from each patient's individual baseline data. By selecting the 17 standardized clinical variables as input into the NPS analysis, we also can expect minimal experimental bias in patient characterization in different regions. Conceptually, an important novelty of the NPS approach is in converting the stage characterization, conventionally performed by using the specific values of expert-selected variables, into objective determination of the same physical quantity for any patient with an HCC diagnosis. Consequently, NPS delineated the same phenotypes in the Turkish HCC patients (as it will for any patient cohort with an HCC diagnosis), just as it did in Italian HCC patients. Thus, the quantitative ordering of the NPS diagnosed HCC stages according to their characteristic τ_s permits the study of the τ_s- dependent trends of characteristic clinical variable values without the need for longitudinal data. In this sense, the limited possibilities of future biology of an individual patient's tumor is already encoded in the clinical data from the baseline visit.

We report here (Fig. 9b) that the Turkish patients were diagnosed only in a smaller subset of 16 out of the 25 HCC phenotypes that were populated by the Italian patients (Fig. 9a). These 16 phenotypes, populated exclusively by 100% of the patients in the Turkish cohort are also populated by Italian patients, but these represent only 20-30% of the patient distribution in the Italian cohort.

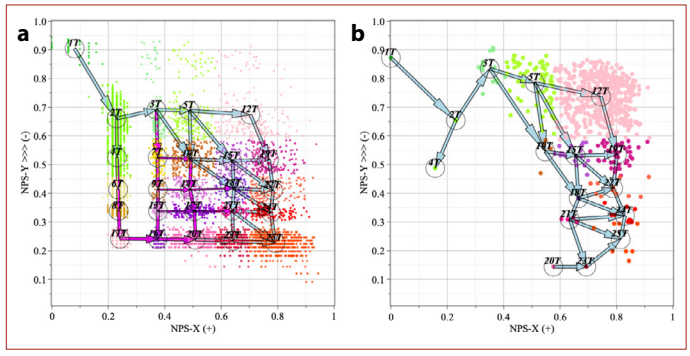

Figure 9. (a) Time-dependent progression between the 25 diagnosed stages for both Italian **(a)** and Turkish **(b)** cohorts. **(a)**, Arrows indicate directions and possible HCC progression paths through NPS identified stage phenotypes sT for Italian patients. Magenta arrows show paths exclusive to the Italian cohort, while gray arrows indicate shared paths for both Italian and Turkish patients. **(b)**, Arrows indicate directions and paths of HCC progression through NPS identified storage phenotypes sT, exclusive to Turkish patients.

Figure 9 shows all possible time-dependent progressions between the 25 diagnosed stages for both the Turkish and Italian cohorts, with the arrows pointing from earlier to the later NPS phenotype in a time-ordered progression. In Figure 9a we show by magenta arrows the model of HCC progression through thew phenotypes, which are exclusive to the Italian population, which we reported previously (2). We have shown that the clinical interpretation of HCC progression of majority of Italian patients (along the magenta arrows) is a dominantly tumor biology-driven process (quantified by the magenta arrows parallel to NPS-Y axis), occurring within a constant extent of liver microenvironmental damage (quantified by the down-oriented magenta arrows parallel to NPS-X axis). In contrast, in the phenotypes shared by the minority of Italian patients and the majority of Turkish patients, the HCC progresses by a more complex disease burden generating mechanism, consisting of simultaneous tumor biology-driven damage, accompanied by advancing liver microenvironmental impairment, i.e. the disease progresses by a more complex mechanism, consisting of simultaneous tumor biology-driven and also by liver microenvironmental (inflammatory) processes. This is represented by the progress along the "diagonal" (light gray) arrows, whose angle and length quantify the extent of simultaneous progression of both factors.

We consider possible reasons for these different findings in the 2 cohorts. The Italian patients were diagnosed predominantly through surveillance of those patients who were known to have chronic hepatitis and were thus considered at risk for HCC development. The Turkish patients had much lower surveillance and as a probable consequence had more advanced HCC stage at diagnosis and thus a

poorer survival in their non-transplant patients. Another reason may be the institutional and treatment-based preselection of patients. This may be related to clinical practice in the healthcare facility, to demographics and to etiology, as there was more HCV in the Italian cohort and more HBV in the Turkish cohort. The Turkish patients also had a greater incidence of males and were typically of younger age.

NPS phenotypes are time-ordered by HCC progression stages, and consequently by the increasing burden of the disease. While Italian patients covered the whole progression interval by being discovered both early, in the middle and also in the final stages of HCC progression dynamics, Turkish patients were represented by the patient sub-group which comes to clinic predominantly in the middle of the progression pathway of the HCC, since they are mostly in phenotype 12T, which is just in the middle of the biological τ_s interval for HCC. We know from the full Italian HCC cohort characterization (2), that 12T is a phenotype in which the patient data shows there to be a simultaneous and quantitatively balanced presence of networked biomarkers of early as well as late HCC stages. In other words, patients in 12T have complex biology and are just in the progression state of HCC when large disease burden biology starts to contribute equally as the early stages with a lesser disease burden biology. So, it is just the time in the disease history, when a clinician might recognize that there is a problem. The ethnic and comorbidities context in the Turkish cohort is what leads to mixed HCC biologies and which also excludes the "simpler" phenotypes, seen in the Italian (but not in the Turkish cohort), since in the Italian cohort only one biology (either early decrease in early stages or late increase in later stages) determined the HCC progression. By contrast, the Turkish patients have both stages changing simultaneously (see Fig. 9b).

Our NPS strategy also led us to observe that in the majority-HBV etiology of the Turkish patients, HBV incidence in HCC patients was maximal at the early HCC stage patients and decreased proportionally with HCC progression (Fig. 4). We consider that this might be explained, either by the HCC growth, initiated within the liver environment already affected by HBV, which in the Turkish cohort is diagnosed practically for all patients in these sT stages, is more extensively destroying parenchyma and thus patients die before their HCCs can get too large, that is a liver death.[9] Alternatively, we consider that the HCCs start to grow only when the HBV disease becomes more advanced (hence the years-long latency between HBV infection and HCC diagnosis), so that these early cancer stage patients die from a hepatitis/liver failure death before the HCC can grow too large. Patients with hepatitis B surface antigen are typically under close follow up and so are more likely to be diagnosed with smaller size tumors, although the literature is mixed on this point.[10-12] This increasing frequency of deaths, caused by the progressing impact of death risk factors in early sT stages, leads to a depletion of the HBV-positive patients from later (t>s) T stages. This results in dominance of cancer biology in defining the clinical profiles and outcomes of patients, which were diagnosed by NPS in those later HCC stages.

Our approach also allowed us to identify 2 treatment-related groups (Fig. 8), and make 2 observations. Firstly, that in four T phenotypes 3T, 5T, 19T and 24T, there was a 2.7 to 3.5 times longer typical length of survival for the transplanted (T) than the non-transplanted (nT) patients who had clinically identical phenotypes (red in Fig. 8a). By contrast, for a separate set of five T phenotypes (2T, 12T, 15T, 22T and 25T), patients (Fig. 8a insert) had only 1.7 to 2.2 longer typical length of survival than clinically identical nT phenotypes (blue in Fig. 8a). Furthermore, in a second observation, we found that some phenotypes benefit more in survival after transplant, even though they all fulfilled the current inclusion criteria for liver transplantation (compare survival for 24T versus 19T patients in the red trend of Fig. 8a). These can only be found after the identification of the 25 phenotypes discovered by the NPS strategy, that cannot otherwise be identified by current standard clinical approaches. As observed elsewhere, only a minority of newly presenting HCC patients are eligible for curative therapies under current guidelines,[13, 14] although the survival differences between surgical and non-surgical treatments for small HCCs can be minor.[15, 16]

Our working hypothesis to explain these results is that for all Turkish Inonu patients, their primary disease is the liver damage, which leads to the (secondary) appearance of HCC. In addition, the damaged liver micro- and macro-environment of HCC tumors in Turkish patients strongly influences the topologies of the relationship networks between the coherently observed values of the 17 variables. This leads to significantly worse survival in the Turkish patients, compared to the surveillance-collected Italian cases.

Conclusion

We show that HCC in Turkish and Italian patients is likely driven by differing processes, the former more complex than the later, even though the Turkish cohort (mainly 12T phenotype) does not catch the whole spectrum of phenotypes (1T to 25T) as found by the nation-wide surveillance strategy in Italy. The fact that only a subset of all HCC phenotypes appears in the Turkish cohort (12T) may be explained by the non-screened HCC patients coming with HCC as a secondary problem, and is primarily driven by the liver environment, which behaves differently to the Italian situation, where HCC is (predominantly) in healthier livers (only ~ 30 % of Italian patients might have the similar environment). Specifically, the Turkish HCC patients are not diagnosed with pheno-

types *6T, 7T, 8T, 9T, 11T, 13T, 14T, 16T, 17T*, which, in contrast are heavily populated by Italian patients.

Note 1. *We made our NPS staging of HCC available through the web tool residing at https://apkatos.github.io/webpage_nps. Accessing the URL provides extension to user local web-browser, so all data handling and processing is done locally at user's computer, without collecting any information or data.*

Disclosures

Ethics Committee Approval: This work complies with the guidelines of the World Medical Association, Declaration of Helsinki. It was approved by the Non-Interventional Clinical Research Ethics Committee of İnönü University (Institutional Review Board Approval No. 2024-6196).

Informed Consent: A waiver of written informed consent was granted in accordance with local guidelines, as the study involved de-identified data and mostly deceased patients; therefore, informed consent was not obtained.

Conflict of Interest: The authors declare no conflict of interest. All authors have read and agree with the contents of this paper.

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