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Accumulated Laboratory Data in B12 Vitamin Blood Level Time Dependency Studies in Patients with Myeloma, Lymphocytic Leukemia and Myeloblastic Leukemia in Latvia

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Abstract. **Vitamin B12 blood level in patients with myeloma (C90 - International Classification of Diseases (ICD-10)), lymphocytic leukemia (C91) and myeloblastic leukemia (C92) prior and after the diagnosis and also BCR-ABL (fusion gene from *breakpoint cluster region BCR* gene and *tyrosine-protein kinase ABL1* (Abelson murine leukemia) gene) tests for C92 patients were studied.**

Clinical records of 20 C92 patients in Riga East University Hospital were complemented with 6987 B12 clinical test data accumulated in E Gulbis laboratory (EGL) for 7451 patients over 20 years period. BCR-ABL and B12 dynamics for 11 patients with sufficient number of BCRABL and B12 tests were studied.

Oracle Cloud with pseudonymized data replica from more than 350 000 000 original EGL clinical test data was used. The data were selected by online analytical processing and SQL built in tools and then used in offline analysis and visualization.

Annually there are 107, 189 and 91 confirmed cases of C90, C91 and C92 in Latvia. EGL has 30% more C90-92 patients, due to suspected but later unconfirmed cases. Out of 7451 patients 1386 had one B12 test, two- 548, three and more- 864. The patients with diagnosis fluctuating between C90, C91 and C92 were excluded from the study. The data for the time period of 10 years before and after the first diagnosis were analyzed.

Results. Methods and tools for data extraction and analysis from large amount of archived clinical test data were developed and applied. High and very high B12 level was observed for 53% of C92 patients starting from 3 years prior to diagnosis. For C90 and C91 patients B12 level changes around the diagnosis date were also observed although the effect was considerably smaller. Analysis of 11 selected patient data with clinical records showed timewise correlation between B12 and BCR-ABL for 3 of the patients.

Keywords: B12 vitamin, myeloma, lymphocytic leukemia, myeloblastic leukemia, laboratory data.

I. Introduction

Medical research usually is time consuming and often involves relatively small groups of persons to be studied. Apart from case studies and larger epidemiologic studies typical research has few dozens and up to few hundred persons studied and involves extensive field work to select and recruit the patients. Advancement of IT technology together with the development of large clinical laboratories has a potential to change the part of the medical science that works in harmony with the results of clinical analysis. Use of IT allows to scan through large amount of accumulated patient data, screen and select the

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data relevant for the chosen research topic. As a result it speeds up medical research and enables better understanding of the connection between different medical conditions and the laboratory test results and reduces the time and resources needed to generate new knowledge. Description of mathematical methods used for extraction of reference intervals from accumulated data are in [1].

In this work a technology designed to extract and analyze anonymized data from large clinical laboratory test result database that is representative of the whole population of Latvia was used. The technology has been developed in Egils Gulbis Laboratory (EGL), Riga, Latvia. There are several challenges in working with accumulated data like data protection, potential data leaks, patient selection for analysis and speed of data processing.

Study of B12 vitamin in Latvia population was selected as the test playground for this technology.

In this work a large amount of clinical test data accumulated in EGL over the period of more than 20 years was analyzed. Study includes results of the vitamin B12 blood level over time period of 10 years prior and after the first diagnosis for patients with myeloma (C90 - International Classification of Diseases (ICD-10)), lymphocytic leukemia (C91) and myeloblastic leukemia (C92). The results of this in silico study were complemented with a limited study of clinical records of 20 patients selected from the cohort of 60 patients from Riga East University Hospital. The clinical record study also includes analysis of BCR-ABL fusion transcript results for C92 diagnosed patients.

The diagnosis group C92 is subdivided in following groups: C92.0 (acute myeloblastic leukemia), C92.1 (chronic myeloblastic leukemia), C92.2, C92.3, C92.4, C92.5, C92.6, C92.7, C92.8, C92.9 [2]. Publicly available statistics in Latvia distinguishes only C92.0 and C92.1 subgroups of C92 diagnosis [3]. All other data are not differentiated under C92 group of diagnosis. In this work the diagnoses C90, C91, C92 were used since subgroups are not always defined for laboratory patients.

The 92.1 diagnosis is usually confirmed by combination of karyotyping and / or FISH and complete blood count. In the case of positive BCR-ABL fusion transcript, specific tyrosine kinase inhibitors are prescribed for treatment. Therapy efficiency and disease progression are then monitored with BCR-ABL Real-Time Quantitative Polymerase Chain Reaction (RT-Q-PCR or PCR) test [4], [5].

In less than 10% of patients with chronic myeloblastic leukemia (CML), the Philadelphia chromosome (Ph) is not identified, but there is presence of the associated BCR-ABL molecular abnormality (Ph-negative, BCR-ABL-positive CML) detectable by PCR [4], [6], [7]. Up to 5% of all CML patients are BCR-ABL negative [8]. BCR-ABL negative and BCR-ABL-positive CML patients differ by clinical manifestations such as age, leucocyte count, monocyte count, basophil count, percentage of blasts in peripheral blood, response to chemotherapy and survival [7], [9], [10]. Ph-negative, BCR-ABL-positive CML patients usually have no other chromosomal abnormalities prior to diagnosis, but approximately 30 % BCR-ABL-negative CML patients have an abnormal karyotype at the time of diagnosis [8], [9].

Since B12 is not synthesized by human tissues the blood level depends on the dietary and intentional intake of the vitamin. The dependency of the B12 level on lifestyle, dietary patterns and B12 intake should be taken into account when interpreting B12 data. Elevation of B12 blood level can be divided into elevated and significantly elevated. Significantly elevated B12 level is defined in this study as more than 2.5 times higher than of reference interval (RI). There are different medical conditions related to these two groups of elevated B12 levels. Moderate elevation of B12 among other causes can be related to kidney and liver damage that can also be a side effect of oncological treatments.

There is a number of reports that links significantly elevated B12 level with some medical conditions for example chronic myeloid leukemia [12],[13], [14], [15], hepatocellular carcinoma [14], [16], [17], polycythemia vera [15], [16], chronic myelomonocytic leukemia [16], primary hypereosinophilic syndrome [7], [15], promyelocytic leukemia [16], myelodysplastic syndrome [16], primary myelofibrosis[16], acute leukemia [16], liver metastases [16], breast cancer [16], colon cancer [16], cancer of the stomach [16], pancreatic tumours [16], liver disease [15], [16], [17], [18], kidney disease [14], [15], [16], [18], autoimmune diseases [14], [15], [16], bronchopulmonary disease [15], [16], alcoholism [14], [15], [16].

For myeloblastic leukemia elevation of B12 is caused of proliferation of myeloid cells containing B12 bound to transcobalamin I.

It has been reported that B12 can be an earlier marker for some oncological diseases [11]Click or tap here to enter text..

II. EXPERIMENTAL TECHNIQUE

A. EGL clinical test result database

EGL database stores clinical test results for all patients tested in laboratory together with patient basic data like age, sex, affiliation with medical establishment, diagnosis provided by physician for each of the test requests, source of the test request for each of the tests. There are more than 100 000 000 results of clinical test data in the database. Size of Latvia population is 2 million. As EGL serves the whole of Latvia the database is representative for the whole of the population of Latvia. Original patient data are stored in EGL information system based on Intersystems Cache. Full data replication to Oracle Cloud was used for faster and simpler processing. Ability to use Oracle analytic window functions and row pattern recognition allows for more accurate and expressive data selection criteria than basic standard SQL. Replication was built using Oracle APEX REST data service with structure-independent JSON data records, further mapped to native

tables as materialized views for increased performance. Patient identity was replaced by patient number, all analysis referring a particular patient number can be retrieved from the data replica. For work with several data sources like different laboratories or medical record banks an additional tool for data transfer including patient identity and adding data source identity into the aggregated data set would be needed. The data needed for specific tasks were selected by online analytical processing and SQL built in tools and used in offline analysis and visualization.

The data used in this work were from EGL IT system. In the analysis small subset of data was complemented with data from patients' clinical records from hospital.

Several laboratory methods were used over the time for the B12 tests. Prior to data matching the uniformity of the results were tested and no correction coefficients were needed to match the results from different methods. There was only one apparatus performing B12 tests at any given time, so there was no need to use the identity of the apparatus in the data analysis as this information was in unique way related to the time of the B12 test. Batch data for the chemical reagents were not used in this work although the system use the batch data as well.

Adoption of the system to process different data sources with numerical values would require specific data transformation code for each data source to bring the data into unified format and the data processing tool that transforms the data obtained with different methods and apparatus into unified value system for each type of measurement, For B12 vitamin the standard sample test results can differ up to 20% and even more between different laboratories, within the framework of international quality control the coefficient of variance between different laboratories is 15% [19].

Several subsets of data were used for the purpose of this work. One subset was 7451 patients with C90, C91 and C92 diagnosis. Another subset was data of B12 blood level measurements from 132379 patients.

The complete data replica in the Oracle was used to compactify the selected data sets and create data arrays suitable for later analysis in Excel or in any other program the research team members might prefer to work with.

B. Laborathory methods

All analysis was performed in E. Gulbis Laboratory (EGL) – a certified (ISO/IEC 17025, ISO 15189:2013) clinical laboratory (Riga, Latvia), which provides laboratory services in all regions of Latvia. All the testing was performed according to the reagent manufacturers' user manuals.

B12 tests. Electrochemiluminiscence immune - assay's (ECLIA) method [T1] performed on the Cobas e 801 analyzer was used in EGL from December 2017 up to current time. Manufacturer's RI for this method is 197-771 pg/ml. From March of 2012 till December of 2017 the method based on ECLIA [T2]. Manufacturer's RI for this method is 191-663 pg/ml. From January of 2004 till March of 2012 tests were performed on Elecsys 2010 analyzer. Manufacturer's RI for this method is 180-900 pg/ml.

As the part of regular laboratory procedure, the accuracy of B12 tests was regularly checked against standardized samples and the coefficient of variation (CV) defined as the standard deviation (SD) divided by the mean and multiplied by 100 was calculated. For most of the time CV was below 4%.

BCR-ABL tests. EGL uses The Xpert BCR-ABL Ultra to perform BCR-ABL monitoring tests.

The Xpert BCR-ABL Ultra, performed on the Cepheid GeneXpert Instrument Systems, is an in vitro diagnostic test for the quantitative detection of the BCR-ABL1 chromosomal translocation mRNA transcripts (types e13a2/b2a2 or e14a2/b3a2) and the ABL1 endogenous control mRNA transcript in the peripheral blood specimens from patients previously diagnosed with chronic myelogenous leukemia (C92, CML) [10]. Method is automated, quantitative, real-time, reverse transcription polymerase chain reaction (RT-RT-Q-PCR or PCR).

For a positive result the software calculates the % BCR-ABL/ABL (IS) using the equation

%BCR-ABL/ABL IS = EΔCt(ΔCt) x 100 x Scaling Factor (SF) where delta Ct value is obtained from ABL Ct minus BCR-ABL Ct.

For a negative result the software calculates a theoretical BCR-ABL/ABL ratio by subtracting 32 from the test ABL Ct (Test ABL Ct-32) using the same equation. And the given result is BCR-ABL was not detected at a detection limit of %(IS).

The scaling factor (SF) is a lot-specific parameter that is embedded within the test cartridge barcode. The value of this factor and the lot-specific EΔCt are determined in quality control testing of each assay lot using secondary standards derived from the world Health Organization (WHO) international genetic reference panel for quantitation of BCR-ABL transcript. Together, the secondary standards and the lot-specific EΔCt and SF values, calibrate the quantitative output the assay to the IS. The SF value is arbitrarily set for 1.22 for use in the example shown here.

C. Patients

Annually there are 107, 189 and 91 confirmed cases of C90, C91 and C92 in Latvia according to the statistics [3]. EGL data has 30% more patients with C90-92 diagnosis than Latvia statistics, the difference can be attributed to suspected but later unconfirmed cases. No selection was done against these cases. BCR-ABL test results indicate confirmed diagnosis. Out of 7451 EGL patients with C90-C92 diagnosis 1386 had one B12 test, two- 548, three and more- 864. The patients with diagnoses fluctuating between C90, C91 and C92 were excluded from the study (unspecified or unconfirmed specific oncohematologic diagnosis). The data for the time period of 10 years before and after the first diagnosis were analyzed. Out of 60 preselected clinical records of C92 patients in Riga East

University Hospital 20 patients with less "side diseases" and less complicated clinical history were selected for this study. Very few B12 data were found in the clinical records of the hospital while considerably more B12 data were accumulated in EGL database for the same patients, the reason being that patient clinical record at hospital has only the results of the tests done during hospitalization or treatment. The study was approved by Riga Stradins University and Riga East University Hospital ethics boards.

III. Results and Analysis

The number of B12 tests performed by persons with diagnosis C90-C92 is given in fig. 1. There are few tests done earlier than 2 years before the diagnosis. 1 year before the diagnosis the number of tests more than triples and after the diagnosis the frequency of testing increases twenty times. The first statistically reasonable sign of moderate elevation of B12 blood level is observed 1 year before the diagnosis for C90 and C92 and only after the diagnosis for C91.

Patient B12 values were divided into 5 groups – "normal" – within reference interval (RI) 197-771 pg/ml where 95% of healthy individuals are within RI; reduced (100-197 pg/ml) and significantly reduced (<100 pg/ml); elevated (771-1700 pg/ml) and significantly elevated (>1700 pg/ml). Out of all 412966 B12 tests performed by EGL 9,3% had

elevated and 1,3% had significantly elevated B12 level. Since there were very few patients with sufficient number of B12 tests we could not get representative results for the time variation of B12 from the analysis of few individual patients. To get an averaged time dependency of B12 level from the diagnosis date we used an approach where all patient B12 values were analyzed against the time of first C diagnosis. B12 blood level development over the time for the patients can be established by following individual patients and performing many B12 tests at regular time intervals. This approach might work only after the patients' diagnosis is confirmed but not before that date. In our work we assume that large number of patients with few B12 test results distributed randomly over the time can provide averaged tome dependency of

B12 level. Using many patients with few B12 tests for each of the patients would better represent the potential deviations from the main trend that might be missed using only few patients with frequent test results.

For the patients studied the number of B12 tests that falls into each of the intervals were counted for 5 time intervals before the diagnosis and 6 time intervals after the diagnosis in total covering 10 years before and 10 years after the diagnosis. The lengths of the time intervals were adjusted to collect sufficient number of results within each of the intervals.

For elevated B12 values for patients with C91 diagnosis the number of tests within the elevated B12 value corridor did not deviate from 9,3% of the number tests performed. Only for patients with C90 and only at interval 1 to 2 years year after the diagnosis date and for patients with C92 from -1 year to 2 years with respect to the diagnosis date the number of tests exceeded the expected average number by at least 3 standard deviations.

For significantly elevated B12 values, (>1700 pg/ml) shown in fig. 2, the number of tests exceeded the expected number of tests by more than 3 standard deviations in the time intervals -1 to 0 years and 1 to 2 years with respect to the diagnosis date for C90; 3 to 8 years after the diagnosis date for C91 and from -2 years to 5 years for C92.

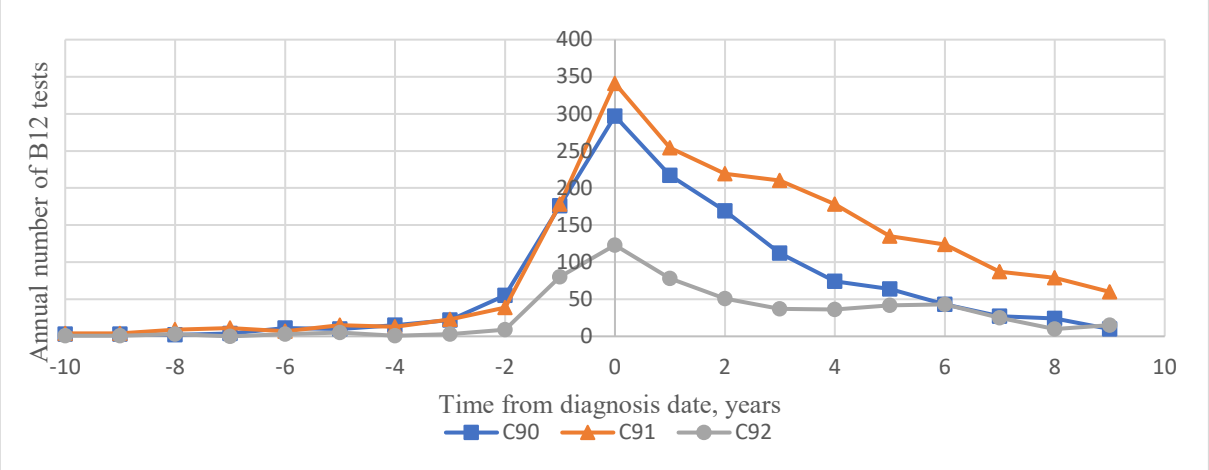

Fig. 1. A2 Annual number of B12 tests performed at EGL for patients with myeloma (C90) -blue squares, lymphocytic leukemia (C91) - orange triangles and myeloblastic leukemia (C92) – grey circles diagnosis prior and after the diagnosis date. . The intensity of testing B12 starts sharp increase 2 years before the diagnosis.

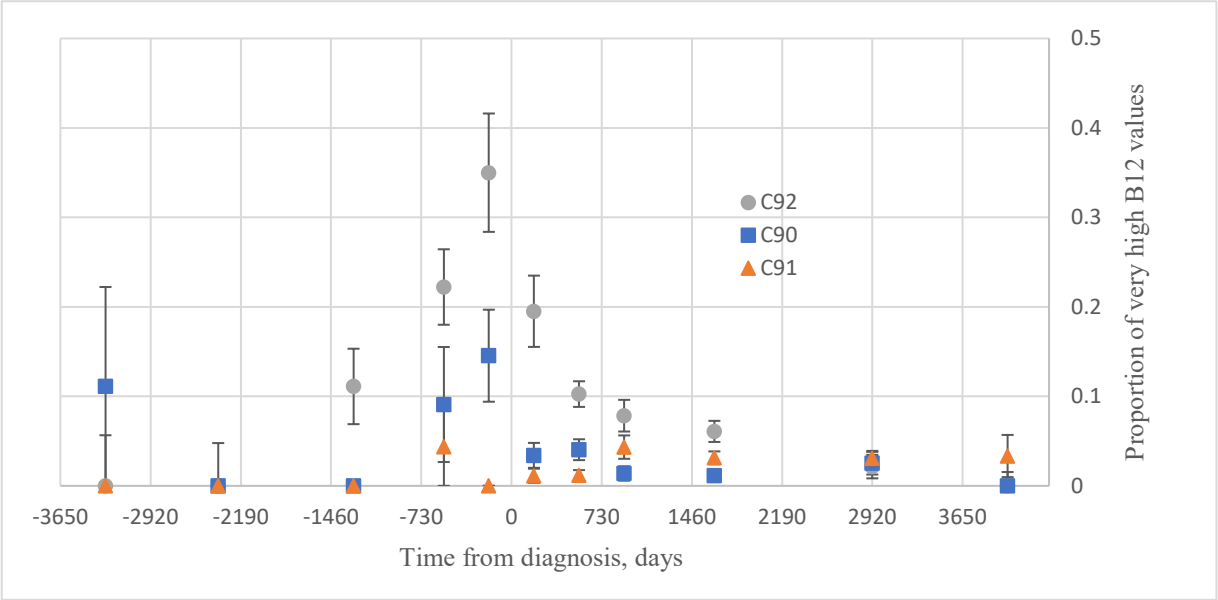

Fig.2. A3, Proportion of significantly elevated B12 test results (>1700 pg/ml) for patients with myeloma (C90) - blue squares, lymphocytic leukemia (C91) - orange triangles and myeloblastic leukemia (C92) – grey circles, in time intervals with respect to the diagnosis date.

These results can be interpreted that for some diagnosis in C92 group it is known that B12 is released in blood and very high B12 indicates that part of the patients have had the disease several years before the diagnosis. For C91 it seems that after remission period a new type of leukemia associated with very high B12 values develops for 3-5% of the patients. Results for C90 are more difficult to interpret.

BCR ABL and B12 dynamics for 11 C92 patients with sufficient number of BCR ABL and B12 tests was studied. Clinical records from the hospital had very few B12 data since only the tests performed while in hospital were kept in the records. The clinical record data were supplemented with the B12 and BCR-ABL data from EGL database. The patients have possibility to perform the tests at other laboratories as well and we do not have access to integrated datasets for the patients. Thus, the clinical test data might be incomplete.

In 3 cases patients had significantly elevated B12 and positive BCR-ABL around the diagnosis time and then both B12 and BCR ABL lowers to normal values in fig.3. This we interpret as the sign of successful therapy.

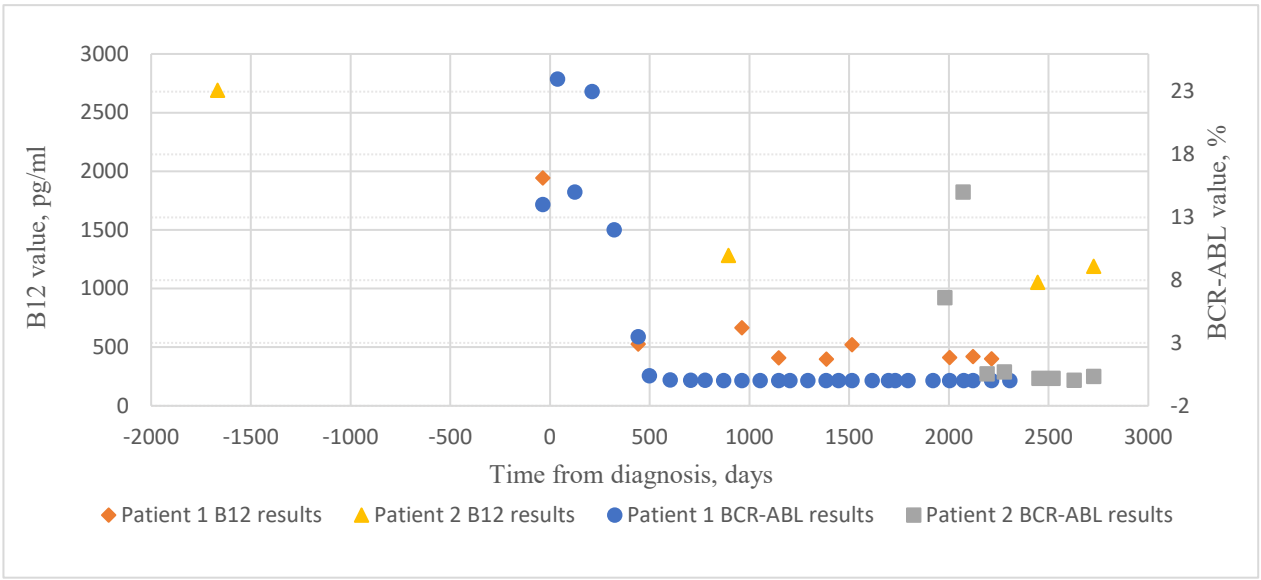

Fig.3. A typical time development of B12 and BCR-ABL test results with respect to diagnosis date. Patient 1 have responded to therapy within 500 days. B12 have returned to reference interval and BCR-ABL is negative. For patient 2 after 5 years BCR-ABL turns to negative, but all B12 tests are significantly elevated

In one case an initial significantly elevated B12 level returned to reference interval value after some time while BCR ABL fusion transcript level was not reduced and B12 again rises to elevated level after 5 years. One patient had significantly elevated B12 that lowered to elevated B12 after 5 years but all 26 BCR ABL tests were negative.

There was timewise lowering of B12 values but no visible timewise correlation between B12 and BCR ABL for 4 of these 11 patients.

Rest of the patients (2) had not sufficient number of BCR ABL data for detailed analysis.

IV. Summary

Methods and tools for data extraction and analysis from large amount of archived clinical test data were developed and applied. With this method the B12 values with respect for the diagnosis data for myeloma (C90), lymphocytic leukemia (C91) - and myeloblastic leukemia (C92) patients were studied. Up to 40% of C92 patients showed significantly elevated B12 in time window -2 to 2 years with respect to the diagnosis date.

B12 tests looking for significantly elevated B12 levels (>1700 pg/ml) can be used as an additional tool to monitor the recovery of patients with C92 diagnosis and can identify suspected cases where BCR ABL tests have become negative after the treatment. Unexpected rise of B12 to significantly elevated values after the successful treatment can be a signal to look for some other diagnosis similar to the one that was cured.

Further developments on the availability of medical records for computerized data analysis together with laboratory test data would open new possibilities in creating new knowledge in interpretation and use of the clinical test data.

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