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Real-world data analysis for factors influencing the quality check status in FoundationOne CDx cancer genomic profiling tests

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Comprehensive genomic profiling (CGP) testing is used worldwide for personalized cancer treatment. Unstained slides from formalin-fixed paraffin-embedded (FFPE) blocks are required for tissue-based CGP testing. However, the use of low quality FFPE specimens can result in testing failure or invalid results. Although several previous studies have indicated that the quality of genomic testing is affected by various factors, the factor(s) that has the greatest influence on CGP testing has not been well studied in real-world data. Thus, we conducted a large-scale multi-institutional study in Hiroshima, Japan to investigate the factors associated with quality check status in FoundationOne CDx CGP testing using real-world data from 1,204 participants from an area with a population of approximately 2.7 million. This study revealed low percentage of tumor nuclei in FFPE specimen, long-term storage of FFPE block, and pancreatic cancer as independent risk factors for qualified status. Of the three factors, percentage of tumor nuclei had the largest effect on quality check status, while the magnitudes of the effects of storage time of FFPE or pancreatic cancer were minor. Collectively, our real-world data indicate that tumor purity is the most important factor for successful CGP, and we would suggest greater than 35% as an ideal percentage of tumor nuclei for CGP submission.

Keywords Cancer genomic profiling, FoundationOne CDx, Quality check, Solid tumors

Cancer genomic medicine enables personalized cancer treatment based on genetic information, which is determined using next-generation sequencing (NGS)–based comprehensive genomic profiling (CGP) tests[1]. CGP tests have been approved for advanced solid tumors in the medical insurance system in Japan since 2019[2]. FoundationOne CDx (F1CDx) and OncoGuide NCC Oncopanel System (NCC Oncopanel) are the first two CGP tests that were clinically approved in Japan in June 2019. Currently, three tissue-based CGP and two blood-based CGP tests are available in Japan.

While whole exon sequencing or whole genome sequencing is expected to be applied for clinical use, CGP tests based on targeted genome sequencing are the gold standard to determine variants in cancer-related genes[3–5]. For targeted sequencing, either amplicon enrichment or hybridization capture-based enrichment is used[3,4]. In general, amplicon enrichment requires less equipment and has a lower cost than hybridization capture-based enrichment, but there are some limitations regarding the small number of targeted regions and difficulty in amplicon enrichment because of the unavailability of specific primers in a certain genomic region. In contrast, hybridization-capture based enrichment is more expensive but can analyze a greater number of targeted regions[4]. F1CDx and NCC Oncopanel are tissue-based CGP tests that use hybridization capture-based NGS to analyze 324 and 124 cancer-related genes, respectively.

Approximately one billion formalin-fixed paraffin-embedded (FFPE) blocks are preserved and archived for clinical purposes worldwide[6]. While the DNA in FFPE specimens is fragmented and chemically modified[7], NGS can be performed with sufficient coverage depth[8], small amplicon size[9], and reduction of sequence artifacts during DNA extraction and library preparation[10]. For clinical tissue–based CGP, unstained sections from FFPE blocks of either surgical or biopsy specimens are submitted to companies that perform the tests. The companies

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routinely extract DNA from the specimens and perform DNA sequencing to call the variants related to cancer development and treatment. However, testing failure occasionally occurs or invalid results are obtained because of poor quality of FFPE specimens. These issues represent significant obstacles to successful CGP testing and delay the identification of personalized treatment by molecular-targeted therapy for cancer patients, resulting in a shorter prognosis[11]. Therefore, the identification of the factors that influence the success or failure of CGP testing is critical to improve patient outcomes.

Previous studies have indicated that the quality of genomic testing is affected by various factors, including cold ischemic time, fixation time, storage period of the FFPE blocks, differences between surgical and biopsy specimens, and inter-institutional differences[12–17]. Additionally, the percentage of tumor nuclei needs to be high enough to fulfill the requirements for CGP testing[18]. Nevertheless, the factor(s) that has the greatest influence on CGP testing has not been well studied in real-world data. One nationwide cancer genome screening project, SCRUM-Japan GI-SCREEN, revealed the impact of DNA integrity, specimen type (surgical/biopsy), primary tumor site, and FFPE block storage period on the success rate of Oncomine Caner Research Panel tests[16]. However, it is still unclear whether these results from amplicon-sequencing NGS panels can be applied to hybridization capture–based NGS panels including F1CDx. The current Japanese Society of Pathology Practical Guidelines[17] have suggested factors of FFPE blocks that affect genetic, genomic, and transcriptomic analyses, but do not specify recommendations for CGP tests.

In this study, we explored the influence of factors that affect CGP results using real-world data from 1,204 participants at Hiroshima University Hospital and the cooperative hospitals, from an area with a population of approximately 2.7 million. We report the association of individual factors with quality check (QC) status in F1CDx and the magnitude of the effect of each factor by multivariate analyses.

Results

QC status in FoundationOne CDx is associated with the success rate of microsatellite status testing

A total of 1,573 patients underwent CGP tests between September 2019 and April 2024 at Hiroshima University Hospital and the eleven cooperative hospitals, and F1CDx accounted for 76.5% of all tests ($n = 1,204$, Fig. 1A). Beside these 1,204 cases, there were 67 cases that failed in F1CDx testing in Hiroshima University Hospital during the investigation period. Of them, 41 cases (61.2% in failure cases) failed in F1CDx testing due to low DNA yield prior to DNA sequencing. Biopsy and surgical specimens were submitted in 33 and 8 cases, respectively. In addition, there were other 16 cases (23.9% in failure cases) that failed in F1CDx testing due to poor QC status. Similar to failure cases by low DNA yield, biopsy specimens were submitted in 12 out of 16 cases. The remaining ten cases (14.9% in failure cases) failed in F1CDx testing due to technical problems, such as detection of secondary DNA signature or DNA contamination from others. According to technical information of F1CDx, DNA less than 50 ng is rejected without proceeding in DNA sequencing, and these data simply suggest us to submit sufficient amounts of specimens for CGP. In addition, we could not measure the magnitude of the effects from other factors such as FFPE storage time and cancer types. Therefore, we utilized QC status in F1CDx (pass or qualified, also refer to Method section) in the following studies, instead of failure cases of testing.

Of the 1,204 cases that underwent F1CDx testing during the investigation period, 1,024 and 180 were determined as pass and qualified, respectively. Analysis of the patient characteristics revealed no statistically significant differences in institute, year of examination, and age and sex of patients by QC status (pass and qualified tests) (Supplementary Fig. 1). We first compared the success rate of microsatellite status testing between the pass and qualified status to measure the clinical impact of QC status in F1CDx, and confirmed the microsatellite status testing more successful in the pass status group compared with the qualified status group (Fig. 1B).

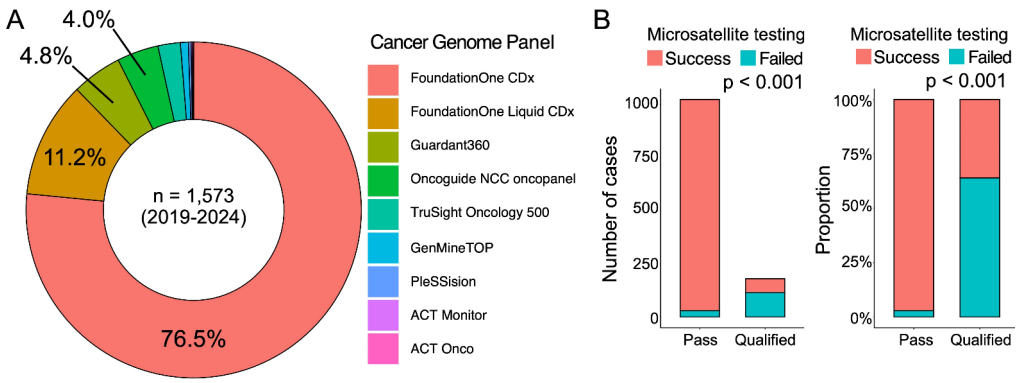


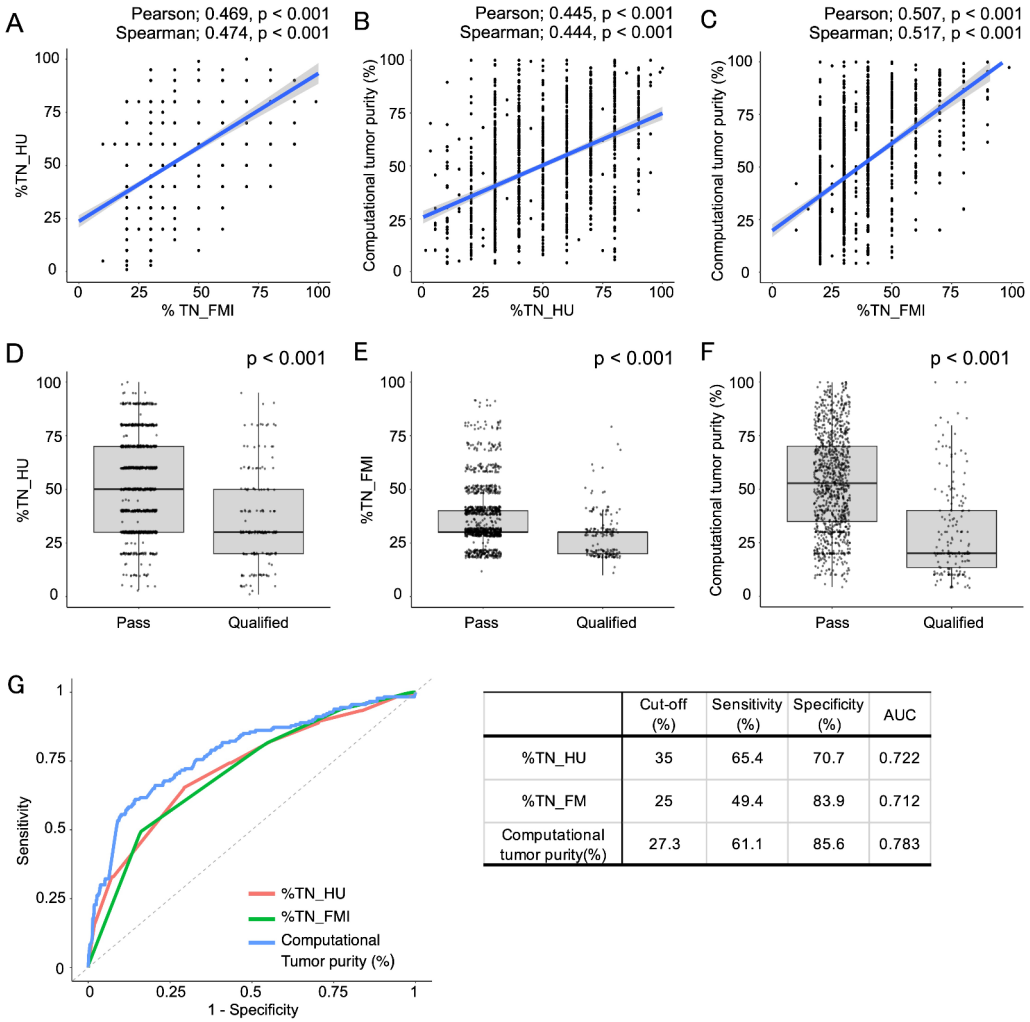
Fig. 1. Association of quality check status in FoundationOne CDx with the success rate of microsatellite status testing. (**A**) The application of comprehensive genomic profiling (CGP) tests in the study cohort. $n = 1,573$. (**B**) Number and proportion of successful and failed microsatellite testing results by quality check (QC) status in the cases tested with FoundationOne CDx. $n = 1,024$ pass cases, $n = 180$ qualified cases.

Tumor purity of specimens affects QC status in F1CDx

We next examined whether the tumor purity of submitted FFPE specimens affected QC status. There were three parameters for tumor purity assessment of the FFPE specimens: percentage of tumor nuclei evaluated by Hiroshima University Hospital and the cooperative hospitals (%TN_HU), percentage of tumor nuclei evaluated by Foundation Medicine Inc. (%TN_FMI, FMI, Cambridge, MA, USA), and computational tumor purity estimated upon DNA sequencing. All three parameters were associated with each other (Fig. 2A-C), and all three parameters were higher in the pass status group compared with the qualified status group (Fig. 2D-F). We next determined the ability of the three parameters for predicting qualified status and found that computational tumor purity estimated upon DNA sequencing was more accurate than the other two parameters (Fig. 2G). Notably, all cutoff values in the receiver operating characteristic (ROC) analyses were approximately 30%, which is the recommended percentage of tumor nuclei in the instructions for F1CDx. Based on these ROC analyses, we determined 35% as a cutoff value of %TN_HU in the later analyses.

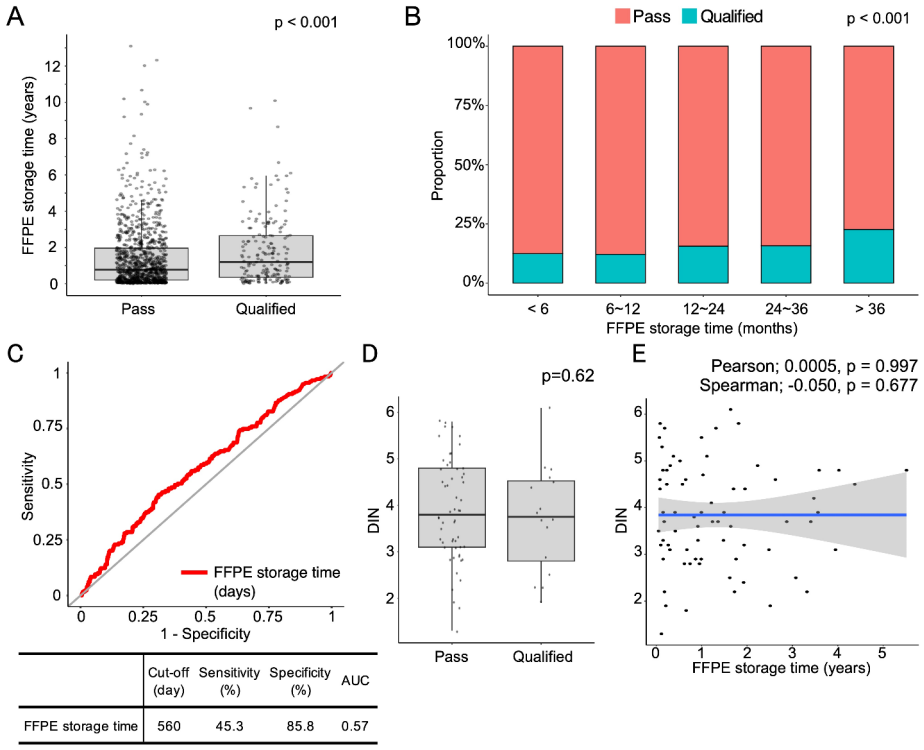
The effect of storage time of FFPE on QC status in F1CDx

We also examined if deterioration of FFPE block because of extended storage time affects QC status. The Japanese Society of Pathology recommends submitting FFPE blocks stored under three years from the harvest time for cancer genomic studies[17]. Notably, in 181 cases (15.0%), the FFPE blocks were older than three years. FFPE blocks were significantly older in the qualified group than in the pass group (Fig. 3A and B). Storage time



	Cut-off (%)	Sensitivity (%)	Specificity (%)	AUC
%TN_HU	35	65.4	70.7	0.722
%TN_FM	25	49.4	83.9	0.712
Computational tumor purity(%)	27.3	61.1	85.6	0.783

Fig. 2. Association of percentage of tumor nuclei and tumor purity with QC status. (**A**–**C**) Linear regression analyses summarized by scatter plots with trendlines (blue) and 95% confidence intervals (grey) of two of the following three parameters: percentage of tumor nuclei evaluated in Hiroshima University Hospital and the cooperative hospitals (%TN_HU), percentage of tumor nuclei evaluated in Foundation Medicine Inc. (%TN_FMI), and tumor purity estimated upon DNA sequencing (computational tumor purity). (**D**–**F**) Comparisons of %TN_HU (**D**), % TN_FMI (**E**), and computational tumor purity (**F**) in the QC groups. (**G**) Receiver operating characteristic analysis of all three parameters for predicting qualified status. $n = 1,024$ pass cases, $n = 180$ qualified cases.



	Cut-off (day)	Sensitivity (%)	Specificity (%)	AUC
FFPE storage time	560	45.3	85.8	0.57

Fig. 3. Association between storage time of formalin-fixed paraffin-embedded block and QC status. (**A**) Comparison of storage time of formalin-fixed paraffin-embedded (FFPE) block by QC status. (**B**) Proportions of pass and qualified cases in FFPE blocks stored for the indicated times. (**C**) ROC analysis of storage time of FFPE block for predicting qualified cases. $n = 1,024$ pass cases, $n = 180$ qualified cases. (**D**–**E**) Analyses in cases in which DNA integrity number (DIN) was measured in Hiroshima University Hospital ($n = 56$ pass cases, $n = 16$ qualified cases). (**D**) A comparison of DIN by QC status. (**E**) Linear regression analyses between storage time of FFPE block and DIN, summarized by a scatter plot with a trendline (blue) and 95% confidence interval (grey). See also supplementary Fig. 2.

of FFPE block was less powerful to predict qualified status than %TN_HU (the area under the curve (AUC) values ; 0.57 vs. 0.722 in Figs. 2G and 3C, respectively).

We also compared DNA integrity number (DIN) with QC status and storage time of FFPE block in 72 randomly selected cases. The results showed that DIN was not significantly different between the pass and qualified groups (Fig. 3D) and did not correlate with the storage time of FFPE block (Fig. 3E). As DIN may be affected by other factors, we explored the association of DIN with other factors such as specimen type (operation vs. biopsy) and %TN_HU; however, there was no difference or correlation in these factors with DIN (Supplementary Fig. 2A and 2B). We also examined DIN in various types of cancers; the results showed variations in DIN by types of cancers (Supplementary Fig. 2C), suggesting cancer type–specificity of DNA degradation.

QC status in various types of cancers

Cancer tissues are composed of cancer cells as well as stromal cells in the surrounding environment[19,20]. The proportion of cancer cells in the entire cancer tissue is tumor purity, which varies among types of cancers[21,22] and may influence QC status. To evaluate if the cancer type influences QC status, we compared QC status across the 29 types of cancers in the study cohort (Fig. 4A). The number of cases with qualified status was the largest in pancreatic cancer, followed by biliary tract cancer. A correlation matrix plot indicating odds ratio between two of any cancer types for QC status showed that qualified status was more frequent in pancreatic and biliary tract cancers than in other cancers (Fig. 4B). To determine whether %TN_HU and storage time of FFPE block correlated with cancer type, we evaluated %TN_HU and storage time of FFPE block in the 29 types of cancers. QC status was correlated with %TN_HU (Fig. 4A and C); storage time of FFPE block also correlated to QC status but with a smaller impact on QC status (Fig. 4A and D). In seven types of cancers, %TN_HU was significantly lower in the qualified status cases than in the pass status cases, while FFPE blocks were significantly older in the qualified status cases than the pass status cases in only two types of cancers (Fig. 4C and D).

QC status and the methods for obtaining specimens

We next compared QC status between surgically excised and biopsy specimens. While biopsy specimens showed shorter storage time of FFPE blocks than surgically excised ones, there was no statistically significant difference

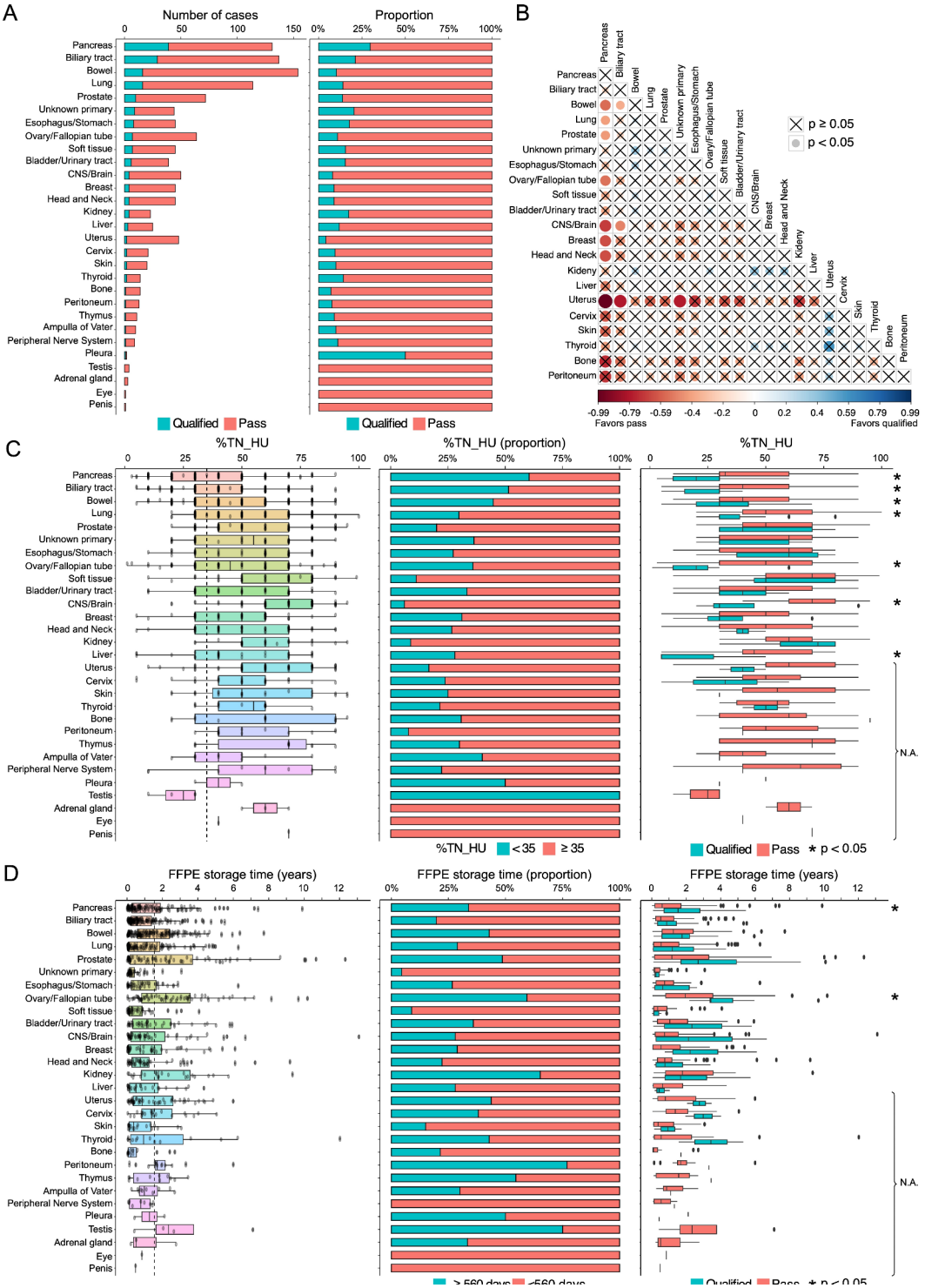


Fig. 4. QC status in various types of cancers. (**A**) Number of cases and proportion of QC status by cancer type. (**B**) Correlation matrix plot of the top 21 types of cancers. The color indicates the odds ratio (row/column) favoring qualified cases. (**C**) %TN_HU by types of cancer. Left: %TN_HU; the dashed line indicates 35%, which was determined as the cutoff value in the ROC analysis in Fig. 2G. Middle: proportion of cases with high and low %TN_HU by the cut-off value. Right: comparisons of %TN_HU between pass and qualified cases. (**D**) Storage time of FFPE block by types of cancer. Left: Storage days of FFPE block; the dashed line indicates 560 days, which was determined as the cutoff value in the ROC analysis in Fig. 3C. Middle: proportion of young and old FFPE block by the cut-off value. Right: comparisons of storage time of FFPE block between pass and qualified cases. $^{*}p < 0.05$. N.A.; not analyzed.

in QC status by the method for obtaining specimens (Fig. 5A-C). We also found that there were variations in the methods used for obtaining specimens by cancer type (Supplementary Fig. 3A). QC status was not different between operation and biopsy in cancers except for pancreatic cancer (Fig. 5D and E). To further explore the influencing factors in pancreatic cancer, we compared FFPE storage time and %TN_HU between specimen types in various cancers (Supplementary Fig. 3B). While we found differences in %TN_HU and FFPE storage time in pancreatic cancer between operation and biopsy specimens, we also observed similar differences in other types of cancers. Currently, the reason for the higher frequency of qualified status cases in surgical specimens in pancreatic cancer is unknown.

Multivariate analysis reveals a major impact of tumor purity on QC status

Our results determined %TN_HU, storage time of FFPE, and pancreatobiliary cancer as factors affecting QC status. We also found differences in QC status in pancreatic cancer regarding the methods used for obtaining specimens. Therefore, we conducted multivariate analysis for the prediction of QC status including these four factors to examine which factor has the greatest effect on QC status. In the multivariate analysis in the overall cohort, low %TN_HU, long-term storage of FFPE block, and pancreatic cancer were determined as independent risk factors for qualified status. Low %TN_HU had the greatest impact on QC status; the odds ratio was approximately two times greater than those for storage time of FFPE block and pancreatic cancer (Fig. 6A). In the comparison of proportion of QC status by a combination of %TN_HU and FFPE storage time, qualified status cases were more frequent in the young FFPE block with low %TN_HU combination than in the old FFPE with high %TN_HU combination (Fig. 6B). In the multivariate analysis limited to pancreatic cancer, tumor purity and methods for obtaining specimens were the factors that affected QC status (Fig. 6C), suggesting

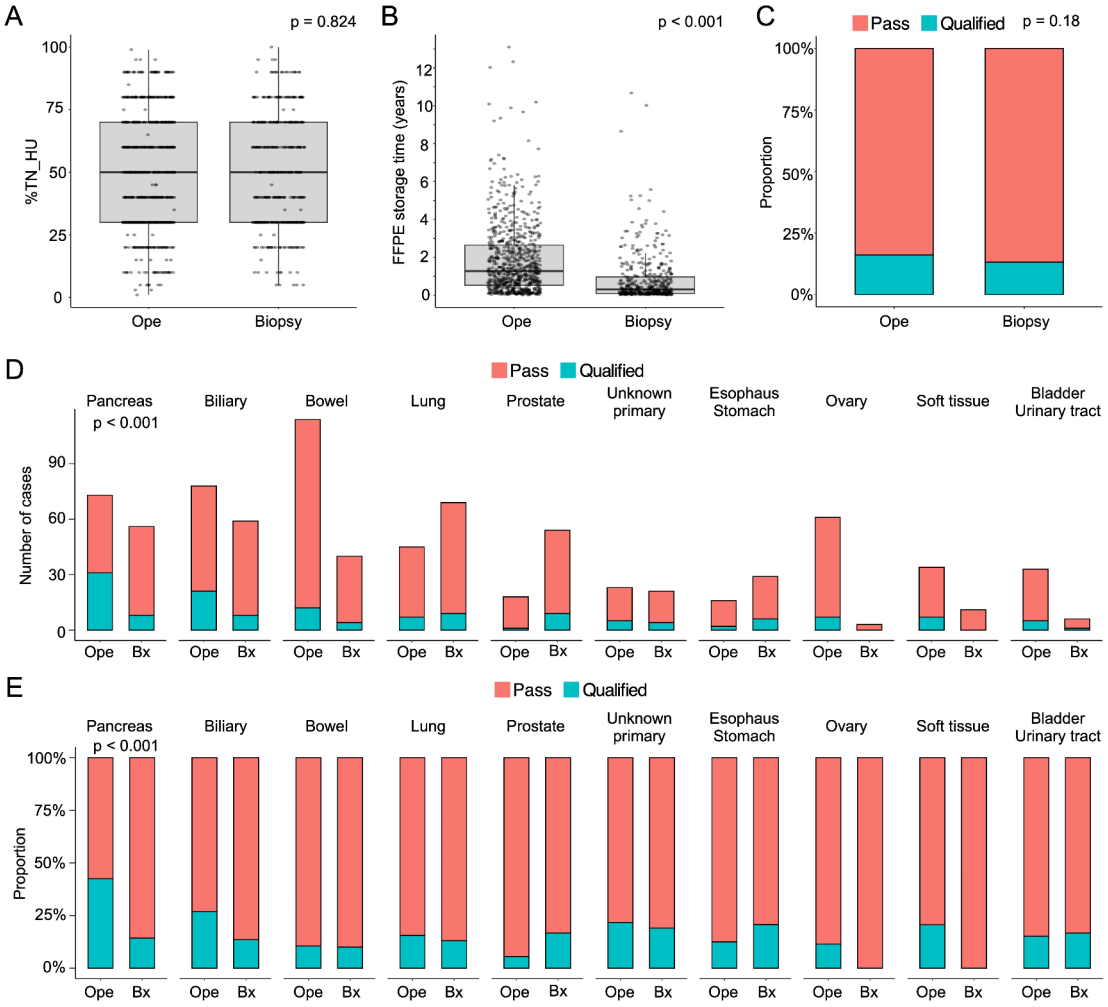


Fig. 5. (**A**–**C**) Comparisons of %TN_HU (**A**), storage time of FFPE block (**B**), and proportion of QC status (**C**) between specimens obtained by operation and biopsy. $n = 753$ operation samples, $n = 449$ biopsy samples. (**D**–**E**) Comparisons of number of cases (**D**) and proportion (**E**) of QC status between specimens obtained by operation and biopsy in several types of cancers. There is a statistical significance in QC status in pancreatic cancer ($p < 0.001$). Bx; biopsy. See also supplementary Fig. 3.

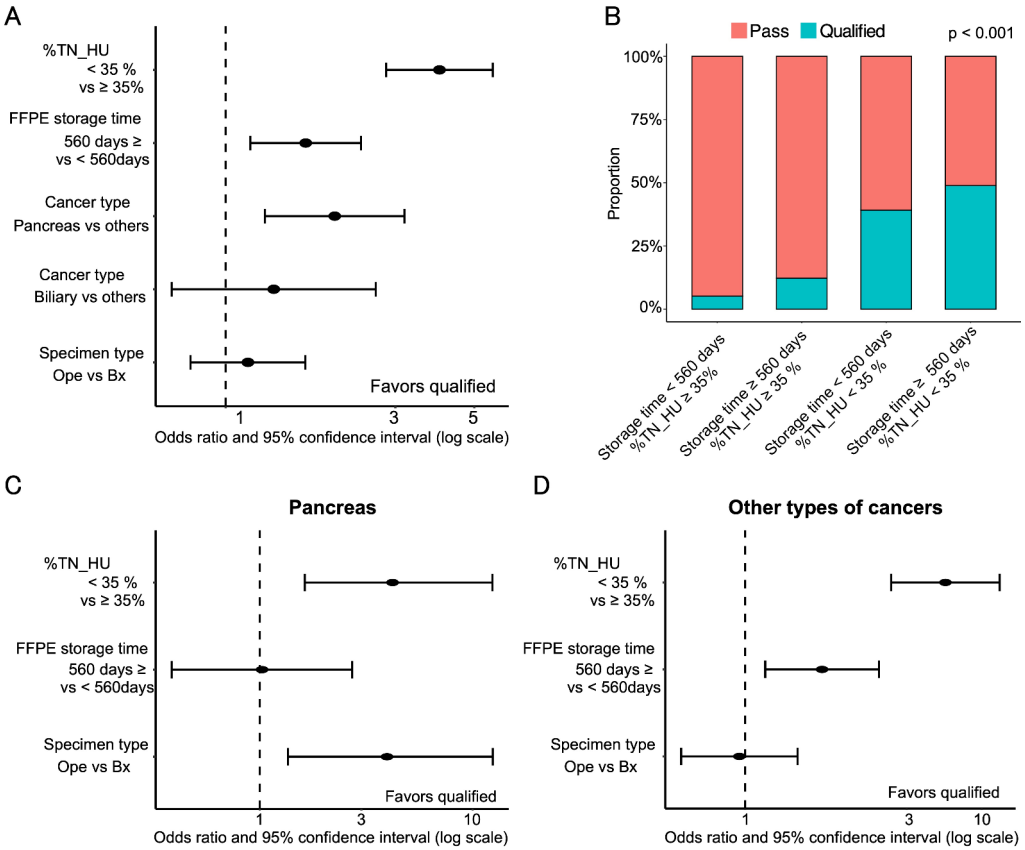


Fig. 6. (**A**) Multivariate analysis was conducted using a logistic regression model to determine risk factors for qualified cases. Data are summarized by a forest plot. (**B**) Comparison of proportion of QC status by a combination of %TN_HU and FFPE storage time. (**C**–**D**) Multivariate analyses were conducted using logistic regression models to determine risk factors for qualified cases in pancreatic cancer (**C**) and other types of cancers (**D**). Data are summarized by forest plots.

whether operative or biopsy specimen affect QC specifically in pancreatic cancers. In contrast, %TN_HU and FFPE storage time affected QC status in the other types of cancer (Fig. 6D).

Discussion

This large-scale retrospective multi-institutional study was conducted to clarify how the quality of FFPE specimens influences CGP testing. Through an investigation including over 1,200 patients, the tumor purity of FFPE specimens was determined as the most influential factor for the validity of CGP testing. Long-term storage of FFPE block was determined as an independent risk factor for qualified status, but the magnitude of the effect was minor. In the analysis by cancer types, qualified status was more frequent in pancreatobiliary cancers, and pancreatic cancer, but not biliary cancer, was determined as the independent factor in a subsequent multivariate analysis. While %TN_HU tended to be low in pancreatic cancer, a multivariate analysis including %TN_HU and FFPE storage time as confounding factors indicated that the pancreatic cancer type was still significant. Currently, the reason remains unclear. In our multivariate analysis for factors influencing QC status in pancreatic cancers, method for obtaining specimens were determined as an independent factor. One possibility is that autolysis of pancreatic tissue in which digestive enzymes are enriched affects the QC status. If it is true, specimens from metastatic lesions might not be affected by autolysis, compared to tumors in pancreatic tissues. Therefore, we reviewed whether primary or metastatic lesions the specimens were from. Nine out of 131 cases (6.9%) were from metastatic lesion, which are not enough to conclude this hypothesis at this time. To address this issue, further studies should examine the time from harvest to fixation and the performance of cold ischemia until fixation.

We compared three parameters for tumor purity and found that they were associated with each other. In our findings, computational tumor purity assessed by DNA sequencing was the most accurate in ROC analyses to predict qualified status. In F1CDx, computational tumor purity is calculated based on copy numbers which are obtained at all exons and genome-wide SNPs upon DNA sequencing. At the same time, these copy numbers are used for QC according to their technical information, although the process in FMI side is not disclosed well. Therefore, low computational tumor purity might be directly related with low QC status. Since the aim

of this study was clarifying how to choose appropriate paraffin blocks for CGP submission, we focused more on in-house H&E evaluation for percentage of tumor nuclei, that is %TN_HU. As reported by FMI, macrodissection is performed depending on whether %TN_FMI is judged to be low. Although this procedure is totally undisclosed to submitter sides, %TN_HU was found comparable to %TN_FMI and enough powerful to predict QC status. In the end of the study, we would suggest greater than 35% as an ideal percentage of tumor nuclei for CGP submission. However, how tumor purity assessment by H&E can be improved to that by sequencing and be generalized in other institutes are issues to be addressed in the future.

While the present study revealed that long-term storage of FFPE block is an independent risk factor for qualified status, DIN was not correlated with either QC status or storage time of FFPE block. These results indicate that storage time of FFPE block had an impact on QC status through factors other than DNA degradation. Considering that %TN_HU had much greater impact on QC status than storage time of FFPE block, this study challenges the significance of DIN examination for CGP. Indeed, there is a report of successful NGS from DNA extracted from FFPE blocks that were up to 18 years old[23]. Therefore, DIN does not always need to be examined before CGP, and tumor purity assessment is more important for CGP.

As our results indicate the importance of tumor purity in CGP, one might wonder if a larger tissue specimen is better for successful CGP. In this regard, surgical specimens would be more successful than biopsy specimens. However, %TN_HU was greater in biopsy specimens, and the storage time of FFPE block was shorter in biopsy specimens. Additionally, there were no differences in the proportion of qualified status cases between biopsy and surgical specimens. These results indicating that the selection of biopsy or surgical specimen does not matter to successful CGP are of great clinical value.

This study has several limitations. First, this was a large retrospective case-control study involving 12 institutes in a specific region in Japan. While our results demonstrate the significance of FFPE block quality on QC status, this real-world data will need to be validated in a nationwide or international collaboration study. Moreover, we focused only on F1CDx, which is the most frequently applied test in our institutes, and therefore our results will also need to be validated in other tissue-based CGP tests. Second, if the FFPE block shows extremely low quality, blood-based CGP would be selected instead of tissue-based CGP. Actually, whether tissue-based or blood-based CGP was selected in this study varied among the institutes (Supplementary Fig. 4), suggesting the existence of this kind of selection bias. Furthermore, in the analysis of failure cases in Hiroshima University Hospital during the investigation period, the major reason of testing failure was low DNA yield from FFPE, presumably too small specimens. In such cases, FMI do not proceed in DNA sequencing, thus, we could not measure the impact from other factors we analyzed in this study. Therefore, we could not conclude whether the effect of low %TN_HU and long-term storage of FFPE block on QC status is directly associated with failure cases. It might be difficult to avoid this selection bias even in a prospective cohort study. Third, there are still some concerns regarding qualified status as a surrogate marker for relatively invalid quality in CGP testing. While we found an association between QC status and the success rate of microsatellite status testing, whether the variant call is also affected in qualified status is not clear. Therefore, we cannot conclude whether old specimens with high tumor purity or new specimens with low tumor purity are more suitable for detecting variants.

Conclusions

Our study indicates that low percentage of tumor nuclei evaluated in H&E slides, long-term storage of FFPE block, and pancreatic cancer are independent factors that predict qualified status in F1CDx. Among these three, percentage of tumor nuclei had the most significant effect on QC status in F1CDx. Based on the current study, we would suggest greater than 35% as an ideal percentage of tumor nuclei for CGP submission, while the magnitudes of the effects of storage time of FFPE or pancreatic cancer were minor.

Methods

Study design and participants

A total of 1,573 patients underwent CGP tests between September 2019 and April 2024.

at the following institutions: Hiroshima University Hospital, Hiroshima Prefectural Hospital, NHO Kure Medical Center and Chugoku Cancer Center, Hiroshima City North Medical Center Asa Citizens Hospital, JA Onomichi General Hospital, NHO Higashihiroshima Medical Center, JA Hiroshima General Hospital, Hiroshima Red Cross Hospital & Atomic-bomb Survivors Hospital, Kagawa University Hospital, Takamatsu Red Cross Hospital, Yamaguchi University Hospital, and Shimane University Hospital. The following characteristics and information were examined in patients who underwent F1CDx testing: sex, age at the time of CGP, institute, cancer type, storage time of FFPE block at the time of specimen receipt by FMI, percentage of tumor nuclei evaluated by H&E staining slides at Hiroshima University Hospital and the cooperative hospitals, percentage of tumor nuclei estimated by FMI, tumor purity estimated upon DNA sequencing, success or failure of microsatellite status testing, and DIN. Over 20% of tumor nuclei is required for submitting to F1CDx, and 50–1000ng of DNA will undergo whole genome shotgun library construction and hybridization capture. If the specimen does not meet these criteria, FMI does not perform subsequent procedures. F1CDx results are certificated as "pass" when all of the following criteria regarding post-DNA extraction analysis fulfills are met: library construction yield ≥ 545 ng, library yield after hybridization capture ≥ 140 ng, median exon coverage ≥ 250x, percent of target > 100x coverage ≥ 95%, and sequencing error rate < 1%. Upon DNA sequencing, a log-ratio profile of the sample is acquired by normalizing the sequence coverage obtained at all exons and genome-wide SNPs (~ 3,500) against a process-matched normal control. This profile is segmented and interpreted using allele frequencies of sequenced SNPs to estimate tumor purity. This copy number profile is integrated in quality check status in F1CDx, although the exact process is not disclosed. If any of these quality control metrics is not met, the

result is returned as "qualified" from FMI. We analyzed the association of pass and qualified QC status with the parameters as described above to determine which parameter(s) affects QC status.

DIN measurement

For the patients who underwent F1CDx at Hiroshima University Hospital, DIN was measured to assess the DNA quality of FFPE specimens submitted for CGP. In brief, DNA was extracted from 10 μM thick unstained sections of FFPE slides using the DNeasy Blood & Tissue Kit (Qiagen, MD, USA). DIN was measured with the Agilent 2200 TapeStation system (Agilent Technologies, Santa Clara, CA, USA).

Statistical analyses

Comparisons among groups were summarized by box-plots with overlaid jitter plots for categorial variables and bar graphs indicating the numbers and proportions of the group for categorial variables using tidyverse package v1.3.2[24]. Statistical tests were performed by Wilcoxon's rank-sum tests for quantitative variables and Fisher's exact tests (binary) or Pearson's chi square tests (more than three variables) for categorial variables. Comparisons between two quantitative variables were summarized by scatter plots with trendlines and 95% confidence intervals. Correlation analysis was performed using Pearson's correlation coefficient and Spearman's rank correlation coefficient with corresponding p-values. Receiver operating characteristic (ROC) curve analyses of tumor purity parameters and storage time of FFPE block for the prediction of qualified status were performed, and results were summarized by ROC curves with AUC values using pROC package v1.18.5[25]. The optimal cutoff values were determined by ROC analyses, and the corresponding sensitivity and specificity were reported. Crude odds ratios between two cancer types for qualified status were summarized by a correlation matrix plot using corrplot package v0.95. Multivariate analyses were conducted using a logistic regression model that included tumor purity, storage time of FFPE block, cancer type, and method of obtaining specimens (operation vs. biopsy). Statistical analyses were performed using JMP Pro 17 (SAS Institute, Cary, NC, USA) and R statistical software version R4.2.2 (The R Foundation for Statistical Computing).

Ethical declarations

This study was approved by the ethical committee for epidemiology of Hiroshima University (Approval number; E2024-0067-00) and the informed consent was obtained from all participants and/or their legal guardian(s). All analyses were performed in accordance with the declaration of Helsinki, and the relevant guidelines and regulations.

Data availability

The data of the current study are not openly available due to the reason of the ethical issue and are available from the corresponding author upon reasonable request.

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Author contributions
H. Niitsu: conception and design of the study; generation, collection, assembly, analysis and interpretation of data; drafting the manuscript. H. Nakahara: collection, assembly, analysis and interpretation of data. K.I. and Y.K.: generation, collection and assembly of data. C.N.H: collection and assembly of data. K.A.: analysis and interpretation of data. T.H.: conception and design of the study; interpretation of data; drafting the manuscript.

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Declarations

Competing interests
The authors declare no competing interests.

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