Predictive molecular pathology in the time of COVID-19

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ABSTRACT

Aims In the time of COVID-19, predictive molecular pathology laboratories must still timely select oncological patients for targeted treatments. However, the need to respect social distancing measures may delay results generated by laboratory-developed tests based on sequential steps a long hands-on time. Laboratory workflows should now be simplified.

Methods The organisation of the University of Naples Federico II predictive pathology laboratory was assessed before (March–April 2019) and during (March–April 2020) the Italian lockdown.

Results The number of patients undergoing single or multiple biomarker testing was similar in 2019 (n=43) and in 2020 (n=45). Considering adequate samples for molecular testing, before the outbreak, next-generation sequencing was mostly used (35/42, 83.3%). Testing six genes had a reagent cost of €98/patient. Conversely, in 2020, almost all cases (38/41, 92.7%) were analysed by automated testing. This latter had for any single assay/gene a significant reagent cost (€95–€136) and a faster mean turnaround time (5.3 vs 7.9 working days).

Conclusion In the times of coronavirus, laboratory fully automated platforms simplify predictive molecular testing. Laboratory staff may be more safely and cost-effectively managed.

INTRODUCTION

In the early months of 2020, COVID-19 has rapidly spread around the world.[1 2] At the time of writing, Italy represents the third country by higher incidence and the second by mortality.[1–3] The public health crisis precipitated in the darkest days of early March, when the Italian government, in the figure of the Prime Minister, locked down the country. People were forced to remain at home; most activities were blocked, including academic teaching and research.[4] Accordingly, all elective medical procedures, scheduled as non-urgent, were postponed. Conversely, it was widely held that the activities directly related to prolong life expectancy of oncological patients must not be delayed. Predictive molecular testing to select potential responsive oncological patients for targeted treatment certainly falls into this category.

The predictive molecular pathology laboratory at the University of Naples Federico II is serving several nearby institutions. During the COVID-19 outbreak, the laboratory kept performing predictive molecular testing on tumour tissue specimens.[5–8] However, laboratory organisation needed to be completely reshaped to limit personnel number and working hours. Before the COVID-19 outbreak, our laboratory adopted, in most cases, laboratory-developed tests (LDTs).[5 6 9–12] Special care had been taken to simplify laboratory workflows. As an example, tissue samples from lung, colon cancer, melanoma and gastrointestinal tumours (GIST) were batched, and all processed by next-generation sequencing (NGS), using the custom SiRe gene panel.[6 9] NGS technology is fascinating and our SiRe assay has recently been adopted by several institutions, along the Italian peninsula.[13] However, NGS requires significant hands-on time, and in some steps, laboratory staff need to work closely. In this current health emergency, however, safety issues are crucial. The number of individuals in laboratory space and physical interactions should be minimised as much as possible, and the protocols should take into account at least the 1 m distance rule. To respect these indications, in our laboratory, a paradigm shift towards simple and fast automated testing platforms was deemed to be not only necessary but also cost-effective.

MATERIALS AND METHODS

Study design

In the times of coronavirus disease, predictive molecular testing for oncological patients is more challenging than usual. Cost-effective and technologically advanced laboratory organisation strategies should be balanced with the need to limit virus transmission. Here, this study aimed to report and to critically analyse the evolving scenario. To this end, all clinical reports issued from 9 March to 20 April 2020 were reviewed. The number of patients referred from oncologists, the number of sample patients evaluated for each biomarker, the testing methodologies adopted, the laboratory staff composition and their working hours, the reagent costs and the turnaround time (TAT) from sample to results were taken into account. In particular, TAT was considered the time between the receipt of samples to clinical reporting. To monitor the impact of COVID-19 on our activity, data were compared with those relative to the same period (9 March to 20 April), in 2019.

Written informed consent was obtained from all patients and documented in accordance with the general authorisation to process personal data for scientific research purposes from 'The Italian Data

Table 1 Sample characteristics in 2019 and 2020 analysis groups

	2019	2020
Total	43	45
Median age (years) (range)	65.7 (39–84)	65.0 (20–90)
Sex (%)–median age (years) (range)	M: 28 (65.1)–67.7 (39–84) F: 15 (34.9)–62.1 (45–74)	M: 28 (62.2)–65.5 (42–88) F: 17 (37.8)–64.1 (20–90)
Adequate (%) versus inadequate samples (%)	42 (97.7) vs 1 (2.3)	41 (91.1) vs 4 (8.9)
Platform, n (%)	NGS, 32 (76.2) Idylla, 4 (9.5) NGS+Tape Station, 3 (7.1) Tape Station, 2 (4.8%) RT-PCR, 1 (2.4)	Idylla, 38 (92.7) NGS, 3 (7.3)
Median TAT (days) (range)	7.9 (3–22)	5.3 (1–12)
Sample type, n (%) - subtype, n (%)	Histological, 33 (78.6) Resection, 24 (72.7) Biopsy, 9 (27.3) Cytological, 8 (19.0) Cell block, 6 (75.0) Smear 2 (25.0) Extracted DNA, 1 (2.4)	Histological, 32 (78.0) Resection, 24 (75.0) Biopsy, 8 (25.0) Cytological, 8 (19.5) Cell block, 8 (100.0) Extracted DNA, 1 (2.5)
Diagnosis, n (%)	Lung ADC, 20 (47.6) Colon ADC, 18 (42.8) GIST, 2 (4.8) Melanoma, 1 (2.4) Cystic teratoma, 1 (2.4)	Lung ADC, 18 (43.9) Colon ADC, 15 (36.6) Melanoma, 5 (12.2) GIST, 2 (4.9) Thyroid anaplastic carcinoma, 1 (2.4)

ADC, adenocarcinoma; F, female; GIST: gastrointestinal stromal tumour; M: male; n: number; NGS: next-generation sequencing; RT-PCR, real-time PCR; TAT, turnaround time.

Protection Authority' (http://www.garanteprivacy.it/web/guest/home/docweb/-/docwebdisplay/export/2485392).

Laboratory workflow and predictive biomarker evaluation

Before the COVID-19 outbreak, the laboratory staff was composed by five units of personnel with interchangeable skills (FP, MR, CDL, AI and UM). Each employee worked full time (35 hours a week). The laboratory director (UM) as a delegate of the head of the department (GT), was responsible of the overall organisation and administration of the laboratory, planning the workload for each employee and organising the staff rotation to harmonise responsibilities and to obtain an adequate TAT.

In table 1, for both periods of time, the patient and sample characteristics are reported. Patients with colorectal cancer (CRC) were genotyped for *RAS* genes, including Kirsten Rat Sarcoma Viral Oncogene Homolog (*KRAS*) and Neuroblastoma RAS Viral Oncogene Homolog (*NRAS*), for V-Raf Murine Sarcoma Viral Oncogene Homolog B (*BRAF*) mutations and microsatellite instability (MSI). In patients with lung cancer, Epidermal Growth Factor Receptor (*EGFR*) sequence alterations were evaluated, together with immunohistochemical (IHC) assessment of gene fusions proteins, including Anaplastic Lymphoma Kinase (*ALK*) and *ROS* Proto-Oncogene 1, Receptor Tyrosine Kinase (*ROS1*). In addition, Programmed death-ligand 1 (PD-L1) IHC was also part of our diagnostic routine. Patients with GISTs were evaluated for Platelet Derived Growth Factor Receptor Alpha (*PDGFRA*) and KIT Proto-Oncogene, Receptor Tyrosine Kinase (*KIT*) mutations, while melanoma cases were evaluated for *BRAF* and *NRAS* alterations.

A number of different genotyping technologies have been validated in our laboratory to ensure a versatile approach tailored on each sample feature. Briefly, the SiRe panel-based NGS assay on Ion S5 System (ThermoFisher Scientifics, Waltham, Massachusetts, USA) is the key laboratory methodology covering 568 hotspot clinical relevant mutations in six genes (*EGFR*, *KRAS*, *NRAS*, *BRAF*, *KIT* and *PDGFRA*).[6 9] The analysis for MSI, an agnostic biomarker for different tumour types, is usually carried out by using a PCR-based Bethesda panel combined with a microfluidic platform (Tape Station 4200; Agilent Technologies, Santa Clara, California, USA) analysis.[10] Only, in selected cases, when biomarker assessment is urgently required in acute deteriorating patients, the fully automated real-time PCR (RT-PCR) platform Idylla (Biocartis, Mechelen, Belgium) is preferred.[11 12] Finally, IHC analysis were carried out to evaluate *ALK* gene fusions by using D5F3 (Ventana, Roche Diagnostics, Basel, Switzerland) and *ROS1* gene rearrangements, by using D4D6 clone (Cell Signalling Technology, Leiden, The Netherlands). In addition, PD-L1 expression analysis in patients with advanced-stage lung cancer by Ventana PD-L1 SP263 Assay on Benchmark XT (Roche Diagnostic) was also carried out.[5]

RESULTS

Laboratory workflow and predictive biomarker evaluation

During the COVID-19 outbreak, the laboratory director, following the head of department recommendations to limit as much as possible the time spent by each employee in the hospital, organised a rotation involving the three biotechnologist units (FP, MR and CDL) to ensure that the genotyping assays for the assessment of *RAS*, *EGFR*, *BRAF*, *KIT*, *PDGFRA* and MSI status would be carried out. A pathologist assistant (AI) ensured the histological processing of the tissue samples. AI was the bridge between the pathologists (CB and EV) assessing cellular content and the predictive molecular pathology laboratory. Overall, biotechnologists (FP, MR and CDL) were organised to follow a weekly rotation, while the assistant pathologist (AI) was asked to attend the lab on alternate days. UM kept working full time to supervise the activities to ensure the reagent supply and to interpret and report results.

The number of patients undergoing biomarker testing in 2020 (45) was similar to that of the same period of 2019 (43). The patient and sample characteristics are reported in table 1. Molecular analysis was successfully carried out in 42 (97.7%) out of 43 samples and 41 (91.1%) out of 45 samples for the 2019 and 2020 group, respectively. The adopted molecular platforms differed (figure 1); the vast majority of cases in 2019 were analysed by NGS (32/42, 76.2%), followed by Idylla (4/42, 9.5%), NGS plus Tape Station (3/42, 7.1%), Tape Station alone (2/42, 4.8%) and conventional RT-PCR (1/42, 2.4%). During the outbreak, to allow laboratory staff rotation, the genotyping laboratory workflow was completely reshaped, and the fully automated platform was prioritised to minimise biotechnologist hand-on times. Thus, in 2020, almost all cases were analysed by Idylla (38/41, 92.7%), and the remaining cases were analysed by NGS (3/41, 7.3%). The results, in terms of type and frequency of detected alterations, relative to assays performed, are reported in online supplementary table 1. As far as predictive IHC is concerned, no differences were reported in the methodological approach between the two periods, and the relative results are reported in online supplementary table 1.

The mean TAT, from the receipt of suitable samples by our laboratory to reporting the results to the clinical care team was 7.9 working days (ranging from 3 to 22) in 2019, while it was shorter in 2020 (5.3 days (ranging from 1 to 12)) (figure 1). Regarding the average reagent costs, NGS required €98 to cover all the reagent cost necessary for nucleic acid extraction, gene panel, library preparation and sequencing for each patient. The

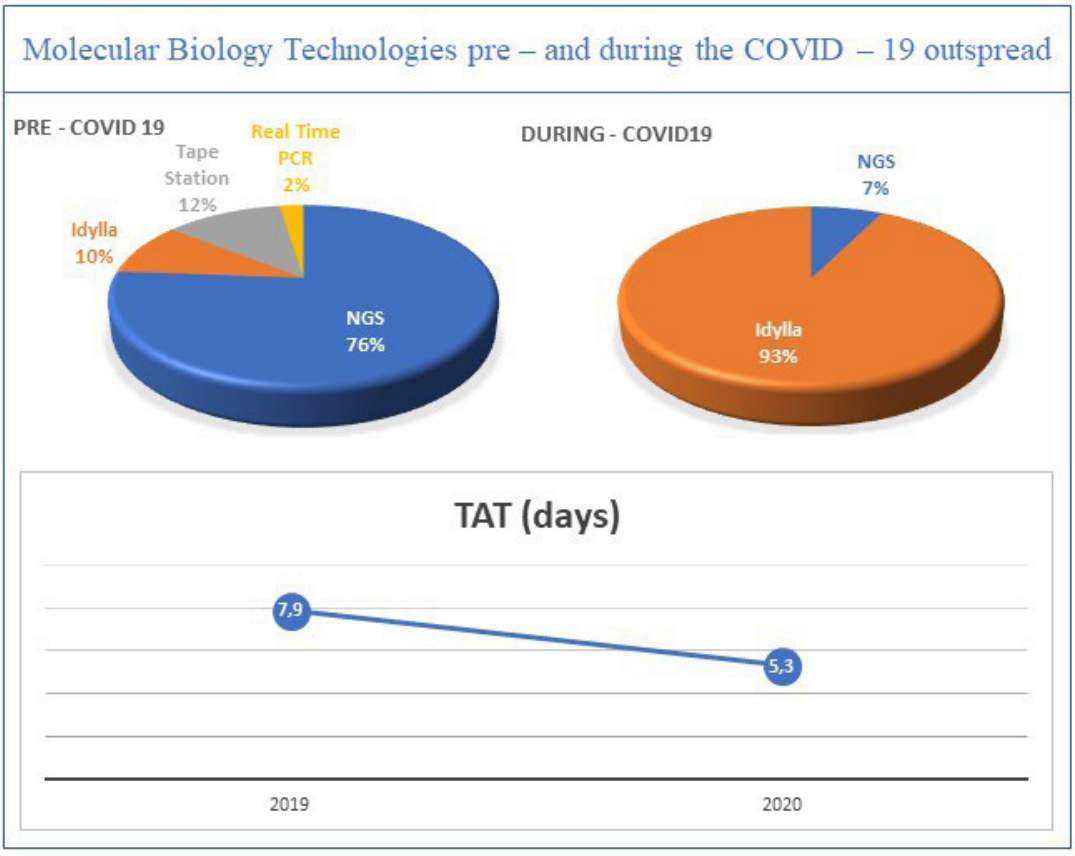

Figure 1 Molecular biology technologies pre-COVID-19 and during the COVID-19 outspread. With respect to 2019, during the COVID-19 outbreak, almost all cases were analysed by automated testing (Idylla), with a reduction of turnaround time (5.3 vs 7.9 working days). NGS, next-generation sequencing; TAT, turnaround time.

reagent costs for the Idylla platform ranged from €95 to €136 for single gene/assay evaluation.

DISCUSSION

During the COVID-19 outbreak, in our laboratory, oncological patients have timely been tested for targeted treatments. In addition, actions were needed to ensure laboratory staff safety. To accomplish these results, laboratory organisation had to be deeply reshaped.[14] Thus, the COVID-19 emergency is also changing the way we practise predictive molecular pathology. Since this activity is crucial to extend and improve the life expectancy and quality of oncological patients, it is not surprising that the molecular testing volume was steady, in contrast to our recent report showing a reduced number of samples in anatomical pathology laboratories.[15]

Fully automated technologies are advancing at a rapid pace.[16] In normal times, this technology has usually been considered as second class, to be 'segregated' in small laboratories featuring limited workload and lacking skilled and trained staff.[17] In larger and academic institutions, as previously reported, this technology was considered only when acute deteriorating patients needed urgent treatment or when nucleic acid quality was unsuitable for NGS.[18] However, in the time of COVID-19, LDTs based on several sequential steps and requiring long hands on time are more difficult to be carried out; fully automated technologies can be prioritised to ensure accurate biomarker evaluation and sustainable laboratory activity. Indeed, we should rethink and re-evaluate the value originally assigned to each laboratory staff working hour, being aware that the time spent by a biotechnologist in the laboratory is today more precious than before.[11 12] In fact, it should be borne in mind that a critical attitude is required to interpret and validate the results. In our experience, the possibility for a highly trained molecular pathologist to visually inspect the RT-PCR curves online, thanks the web-based Idylla Explore application, is crucial.[19] In fact, although most cases (95%) were diagnosed by the fully automated approach only, on some occasions, NGS was still carried out to refine uncertain results displaying RT-PCR curves of undetermined interpretation.[20] Moreover, not all clinically relevant biomarkers are testable by Idylla; as an example, automated assays for *KIT* and *PDGFRA* gene evaluations have not been developed yet. On the other sides, the disadvantage of the fully automated approach is the higher overall cost for a single patient.[21] In fact, the cost of a comprehensive analysis for a patient with CRC (*KRAS*, *NRAS*, *BRAF* and MSI) with Idylla is around €350 with respect to €98 of our SiRe NGS panel. This latter offers also the possibility to simultaneously assess a larger number of clinically relevant biomarkers from the same sample.[22] This is a crucial point in patients with non-small-cell lung cancer where retrospective data can be mined to integrate the *EGFR* analysis with information on *BRAF* and *KRAS* status.[23] Although is not considered a must-test gene yet, *KRAS* is acquiring greater relevance considering the AMG510 (Amgen, Thousand Oaks, California, USA) clinical trials, underlining the role of exon 2 p.G12C point mutation as a positive predictive biomarker.[24]

The need to maintain physical distance between laboratory staff is well established.[25 26] Conversely, since molecular testing is based on formalin-fixed and paraffin-embedded tissue or on ethanol-fixed cytological material, the possibility that the virus would be present in the examined tissue samples is minimal.[27] Conversely, liquid biopsy may represent a potential source of transmission, and recommendations to regulate this activity should be provided soon.

In conclusion, the COVID-19 outbreak underlines even more the concept that predictive molecular pathology should be practised in advanced laboratories by highly trained staff. In fact, even a fully automated procedure needs to be used in the proper scenario, making the versatility of testing technologies a crucial opportunity for modern molecular medicine.

Key messages

- The COVID-19 outbreak modifies laboratory organisation to limit personnel number and working hours.
- Despite these limitations, in our laboratory, oncological patients have timely been tested for targeted treatments.
- This public health emergency underlines even more the concept that predictive molecular pathology should be practised in advanced laboratories by highly trained staff, even if fully automated procedures are employed.

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Contributors UM and GT conceived the study; CDL, FP, MR, RS and MN contributed as biotechnologists; AI contributed as laboratory technician; UM and PP contributed as molecular pathologists; EV, CB, FCS and GT contributed as cytopathologists; UM, FP, PP and GT wrote the paper; all authors approved the final version of the manuscript.

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