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Molecular tests for SARS-CoV-2: data from Liguria Region (Italy)

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Abstract — **The current Covid-19 pandemic makes necessary to identify people affected by SARS-CoV-2. To do this, the most reliable method is the use of the molecular test that is the gold standard to detect positive peoples.**

Here, we provide a comprehensive review on the diagnostic processes through molecular tests for SARS-CoV-2 infection. First, we have obtained information about the testing technologies in the Liguria region's hospitals to find and describe the most common technologies used and to calculate the molecular test's average cost. Second, we have evaluated the sensitivity, the specificity, the safety with respect to the data reported on scientific literature (Real Word Data VS Registrative Studies) and the organizational aspects of the molecular tests.

Clinical Relevance— **This study aims to provide support to the decision makers on clinical, economic, organizational, social and ethical issues related to the use of molecular test for SARS-CoV-2.**

***Keywords*: SARS-CoV-2, molecular test**

I. INTRODUCTION

SARS-CoV-2, acronym from "Severe Acute Respiratory Syndrome coronavirus 2" is a highly transmissible SARS-related coronavirus/SARS-CoV, belonging to the Beta coronavirus type, defined as the causative agent of 2019 Coronavirus disease, known as COVID-19. In December 2019, several patients in Wuhan city, Hubei Province, People's Republic of China, developed forms of pneumonia and respiratory failure similar to the 2003 SARS pandemic [1]. The virus, later called as SARS-CoV-2, despite massive attempts to contain the disease in China, started to spread with exceptional speed and worldwide. Thus, in March 2020, COVID-19 was declared pandemic by the World Health Organization (WHO).

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The new coronavirus spreads from one person to another mainly through droplets containing the virus when people have a close interaction, usually less than one meter [2]. Droplets containing virus can be inhaled or dropped on surfaces exposed to touch of people who can be infected touching their nose, mouth or eyes. SARS-CoV-2 is present in a high concentration in the upper and lower respiratory tract [3]. The virus has been also discovered at lower levels in the kidneys, liver, heart, brain and blood [4]. After an incubation of about 5 days [5], COVID-19 infection shows symptoms like dry cough, fever (about 38° C) and fatigue, often accompanied by loss of taste and smell. Other signs and symptoms attributable to COVID-19, also called clinical criteria, include cold, breathing difficulties, chills, headache, sore throat, sick and diarrhea [6]. The clinical course ranges from completely asymptomatic cases to a rapid devastating course of the disease.

It is therefore necessary to prompt identify, isolate, monitor and treat people affected by SARS-CoV-2. For this purpose the most reliable test, defined as the "gold standard" for detecting a SARS-CoV-2 infection by the Ministry of Health, the Italian Higher Institute of Health (ISS) and WHO, is the molecular test. The Italian Ministry of Health and ISS lists the cases where molecular tests be performed [7]: a) suspected symptomatic case, b) people in quarantine when symptoms appear, c) asymptomatic people, in case of planned hospitalization or imminent access to large confined communities, d) screening of healthcare staff to operate in an high-risk context, e) people under isolation in the way of healing.

Considering the above and with a view of HTA, the paper investigates and assesses the clinical, economic, organizational, social and ethical issues of the molecular test.

II. MATERIAL AND METHODS

A. Material

The sites of the Italian Ministry of Health, the Italian higher Institute of Health (ISS), the Italian drug agency (AIFA), Manufacturer and User Facility Device Experience (MAUDE) and Medicines and Healthcare products Regulatory Agency (MHRA) have important material for our study.

An ISS report [8] gives important indications about the execution of the nasopharyngeal and oropharyngeal swab; indeed, the swab must be therefore made by trained and specialized personnel. According to that report, operators must wear appropriate Personal Protective Equipment (PPE).

The operator must lift the tip of the nose and introduce the swab into the nostril towards the nasopharynx for a length of 8-12 cm. The swab should be gently rotated clockwise and/or counterclockwise and left in place for a few seconds. Then the stick must be inserted, broken in two, in the test tube showing a label indicating: name, surname, date of birth of the patient, date of collection and type of sample.

From information collected by A.Li.Sa. (the Health Authority of the Liguria Region coordinating all the Regional HC Companies and referral Hospitals) about the technologies used for collecting and processing molecular swabs for COVID-19, we know that the regional HC companies and referral hospitals of Liguria can adopt a closed system (a single system in which all the phases of the analysis of the samples take place and that provides the results in about an hour) or an open system (constituted by a set of instruments which perform the extraction of nucleic acids, prepare the reaction mix and perform the PCR and a system where an higher number of operators is required). Moreover, the most used testing technologies are:

- Cepheid's ® closed GeneXpert system: available in 1/2/4/16-module configurations, they all use the same-patented cartridge technology. The cartridge with polypropylene structure is patented, autonomous and disposable. It provides a lot of results in about an hour, including sample preparation. [9]
- Bosh's ® Vivalytic: is an "all in one" solution for molecular diagnostics that allows performing various laboratory tests quickly with a completely automated process. It uses specific cartridges that are scanned, loaded with a sample and inserted into the analyzer box. [10]
- Biorad's ® CFX96: is a powerful, precise, and flexible real-time PCR detection system. It sets up the system quickly, optimizes reactions in a single run, minimizes samples and reagent use, analyzes data faster, uses advanced data analysis tools and configures the system to fit your needs. [11]
- Seegene's ® Nimbus: is an automated workstation that allows the manipulation of liquids from the extraction of bacterial, viral, genomic, parasitic, fungal, and RNA/DNA from various types of samples to the configuration of the PCR. A maximum of 72 samples can be extracted in approximately 3 hours. [12]

B. Methods

First, it has been developed a deep review of the literature available on SARS-CoV-2, the current clinical status, the diagnostic tests available and their clinical and technical characteristics. Based on the clinical practice, to frame a well-structured question is the main key factor of success for an evidence-based decision-making process. To do this we used the PICO model which includes the population being studied (P), the intervention being evaluated (I), the comparator (C) and the outcomes of interest (O). The analyzed Population is composed by patients who need to be subjected to molecular test; the Intervention is the evaluation of molecular test to identify a possible SARS-CoV-2 infection; the Comparison factor is the third-generation antigen test; the Outcome are the evaluation of effectiveness, safety, economic, ethical, legal and social impacts of the molecular test. The research was carried out through a definition of a "search string". More specifically, the search string used is: ["molecular test" AND ("SARS-CoV-2" OR "COVID-19")]. The string was used on PubMed and Cochrane.

With a view of HTA we also analyzed the following domains: [13]

- Health problem and current use of technology (CUR) contain information about the pathologies linked to technology, health problem size, the availability of the technology and the possible alternatives;
- Description and technical characteristics of technology (TEC) contains: the technology in question, its technical characteristics and the reasons why it was developed;
- Clinical Effectiveness (EFF) and Safety (SAF) contain any adverse effects caused by using of the technology and its effectiveness;
- Costs and economic evaluation (ECO) of the technology contains the technology cost analysis;
- Organizational aspects (ORG) contain the organizational aspects related to the use of the technology and the resources that must be activated.
- Ethical (ETH), social (SOC) and legal aspects (LEG) contain social and moral norms, legal aspects and patients' opinion about the technology;

III. RESULTS

The most relevant results are shown below.

A. Effectiveness and Safety

The effectiveness and reliability of the molecular test are described in terms of sensitivity and specificity. Sensitivity and specificity may vary depending on the technology used for processing the samples and for possible errors of the operators: but the sensitivity and specificity of the molecular test for SARS-CoV-2 must be at a minimum [14]:

- Sensitivity $\geq 92\%$
- Specificity $\geq 99\%$

While, for the 3rd generation antigen test, an Italian Ministerial Circular indicates [15]:

- Sensitivity ≥ 80%, but with low incidence contexts ≥ 90%
- Specificity ≥ 97%

As can be seen, the antigen test has lower sensitivity and specificity than the molecular test for SARS-CoV-2, and this is why the molecular test is considered the most reliable diagnosis of SARS-CoV-2 patient's infection; for which, in case of positive antigen test, a molecular test is required for confirmation [15].

Safety of the molecular test. After a deep investigation on the sites of: a) Medicines and Healthcare products Regulatory Agency (MHRA), b) Manufacturer and User Facility Device Experience, c) Italian Ministry of Health (safety warnings section), d) ClinicalTrials.gov, e) Food and Drug Administration (FDA), it has been ascertained that there are no registered adverse effects, on a patient, linked to the use of a certain technology. The sole potential risks associated with the use of the molecular test for SARS-CoV-2 are: i) possible discomfort during sample collection nose/throat swab, ii) possible incorrect test result (false negative or false positive) [16].

B. Economic evaluation

For the economic evaluation, we conducted a cost minimization that shall determine which treatment, between molecular test and antigen test, is the most cost effective. It would be interesting to conduct an evaluation that considers the efficacy / benefit, but in our case, it is too complicated because we do not have sufficient data to evaluate all the consequences of the clinical benefit and so, to evaluate the costs entailed by an extra sensitivity of the molecular test compared to the antigen test.

From the analysis of the single molecular sample costs provided by the each single Ligurian HC Companies coordinated by A.Li.Sa. in January 2020, it emerged that the costs incurred by uses of a closed system are higher than those of an open system. A weighted average is obtained considering the single molecular sample costs provided by the HC Companies and the number of samples processed during each analysis cycle. From the calculation of this weighted average, we can obtain the average cost estimated for a single molecular test for open and closed systems for the Region as follows:

- Average cost for molecular sample for open systems: 16 €
- Average cost for molecular sample for closed systems: 29 €

On the other hand, the 3rd generation antigen test costs about 30 €.

All the above costs include the cost of a single sample and consumables but not of personnel.

Therefore, the molecular test for open systems is economically more advantageous than the 3rd generation antigen test. The cost for a molecular closed system test is slightly lower than the one of an antigen test, even if the two costs are very similar.

It is very important to underline that with an open system it is possible to analyze many samples (about 100) compared to a single antigen test and more. Moreover, with an open system it is used an instrumentation already present in laboratories and therefore, it is not necessary to take into consideration the relevant depreciation.

C. Organizational aspects

Molecular test needs a more complex organization than that the antigen test. In fact, the analysis of molecular tests being carried out in highly specialized laboratories, requires advanced technologies (extraction, Real Time RT PCR), many operators and an interpretation of the results.

D. Ethical and legal aspects

With the appearance of COVID-19, ISS (Italian higher Institute of Health) has deemed appropriate to set up, among the thematic Working Groups for COVID-19, also a Working Group entirely dedicated to Bioethics [1718]. According to the WHO, Health /Authorities /institutions have the duty to develop a local surveillance system that respects certain standards from an ethical point of view: the measures adopted must, anyhow, always proven to be necessary, reasonable, proportionate, non-discriminatory, and transparent.

In emergency situations, and when it is strictly necessary for the protection of the Community, the COVID-19 pandemic control measures taken can impose restrictions on the individual, drawing a new and temporary border to his freedom with the aim to contain the spread of infection.

In the various phases of the emergency, it can be necessary to collect and use personal data of citizens, respecting relevant ethical and legal issues; and always insuring the relevant strict use for the purposes for which they were collected [19]. The technologies and data collection used for COVID-19 do not have, of course, among their objectives, the control of people and their behavior: and shall remain a useful tool for the person and for the Community for containing the infection.

On the other hand, in a context of health emergency, it is of course necessary, to involve the citizens; they must be adequately informed, through timely, reliable and intelligible communications.

In the frame of the ethical aspects, we analyze now also the contact tracing: this procedure consists in identifying and managing people who have been in contact with infected or possibly infected person in the 48 hours prior to the symptoms onset. Also in this case an adequate balance is

always guaranteed among epidemiological effectiveness, respect for the privacy and security of people on all aspects concerning data management.

The Bioethics Committee of the Council of Europe, in a Declaration of April 14th 2020 [20], recalled, among the essential principles in the context of the COVID-19 pandemic, respect for human dignity and human rights "which must guide medical decisions and practices in the context of the current crisis".

The WHO in a 2017 Document entitled "Guidelines on Ethical Issues in Public Health Surveillance" indicates 17 recommendations to promote the ethics of public health surveillance [19].

In conclusion of the Paper, we list here below some important Ministerial Circulars of the Italian Ministry of Health about COVID-19:

- Circular no. 32850 of October 12th 2020 provides indications about the duration and term of isolation and quarantine [21];
- Circular no. 35324 of October 30th 2020 provides indications on criteria for choosing the tests available, for a rational and sustainable use of resources, in different contests [7];
- Circular of November 30th 2020 provides indications on the home management of patients with SARS-CoV-2 infection [22];
- Circular no. 0000705 dated Jan 8th 2021 updates the definition of "COVID-19 case" and testing strategies [23];
- Circular of Feb 5th 2021 provides updates on the use of antigenic and molecular tests for the detection of SARS-CoV-2 [15].

IV. Conclusion and Discussion

With a view of HTA, some assessments of the molecular test have been carried out and this contributes to have reliable and transparent information about the diagnosis process through molecular tests for SARS-CoV-2.

To do the appropriate assessments, it has been used a set of data provided by the Liguria region: the analysis of the technologies for processing molecular samples and the economic evaluation refer to these data. These evaluations anyhow may differ nationally or internationally depending on the choices and needs of Regional HC Companies and referral Hospitals.

In conclusion, the molecular test seems to be the best method for diagnosis; it is more effective, cheaper, safe but organizationally more complex than the antigen test.

A HTA evaluation could be usefull to menage all the dimensions enclosed the organizational ones.

DISCLAIMERS

The authors do not have conflicts of interest to disclose.

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