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BPI

Case Report

A CASE REPORT ABOUT MULTIPLE SUPERNUMERARY TEETH NOT ASSOCIATED WITH SYNDROMES IN A FAMILY WITH A NORMAL KARYOTYPE

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ABSTRACT

The odontostomatologic condition known as hyperdontia is typified by an overabundance of teeth. This case represents a rare form of hyperdontia, with bilateral multiple supernumerary teeth and clear penetration of the phenotypic in the family unit involved in the current investigation. It appears to occur more frequently in patients with hereditary variables regarding this anomaly. The karyotype determination excludes the chromosomal basis pathogenesis. In addition to five impacted teeth (1.8, 2.8, 3.8, 4.7, and 4.8), the 30-year-old patient had an affected supernumerary tooth (distomolar 4.9) when she arrived for our observation. The patient was recommended to permit us to conduct a radiologic screening on his two sisters, who are 13 and 17 years old. Nine impacted teeth were found in the older sister's X-ray photos (1.8 -1.9 - 2.8 - 2.9 - 2.10 - 3.8 - 3.9 - 4.8 - 4.9). In contrast, the youngest sister's X-ray photos revealed four affected teeth: 1.8 - 1.9 - 2.8 - 2.9. The present case study has the potential to serve as a model for evaluating the genetic variables that predispose an individual to hyperdontia and for managing affected families by oral surgery when an odontostomatologic aberration without syndromic manifestations is found.

KEYWORDS: *hyperdontia, supernumerary teeth, impacted teeth*

INTRODUCTION

Hyperdontia is a dental anomaly characterized by extra teeth, whether erupted or un-erupted. This condition can be classified as "real" hyperdontia, where there is an actual increase in the number of teeth, or "false" hyperdontia, which results from a delay in the shedding of deciduous teeth beyond the normal transition period (1).

The nomenclature for these extra teeth was suggested by Tomes, who categorized them into two types: "supplementary" teeth, which have normal morphology, and "supernumerary" teeth, which exhibit morphological and

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volumetric anomalies (2). Supernumerary teeth are further classified by their shape (2)

Hyperdontia is relatively common, with a higher prevalence in males compared to females (approximately a 2:1ratio) (3). It often occurs in individuals with hereditary factors related to this anomaly (4). For instance, a study involving 30 patients with 41 mesiodens found a familial predisposition in 31% of the cases (5). Supernumerary teeth are most frequentlyfound in the upper jaw, particularly in the premaxillary region, accounting for 90-98% of cases (6). These teeth are often impacted (88.7%) and commonly located in the palatine area (7, 8).

Although hyperdontia can occur sporadically, genetic factors can play a significant role, especially in severe cases. It can also be associated with genetic syndromes such as Gardner Syndrome and Cleidocranial Dysplasia, where hyperdontia is part of a more complex clinical picture with additional anomalies.

Early detection of hyperdontia is crucial, mainly because of its potential association with more severe syndromic forms. Early diagnosis can be facilitated by techniques such as G-banding, which involves staining chromosomes to reveal a pattern of light and dark bands. The dark regions are typically heterochromatic, late-replicating, and AT-rich, while thelight regions are euchromatic, early-replicating, and GC-rich (9).

One of the most frequent complications of having supernumerary teeth is dental malposition, which can lead toclinical consequences requiring orthodontic or surgical intervention. Less commonly, impacted supernumerary teeth cancause follicular cysts, neuralgic symptoms, or problems with the eruption of permanent teeth (2,3,10).

Several clinical signs may indicate the presence of supernumerary teeth. One of the first signs is the absence of permanent teeth in the maxillary arch, which could suggest unerupted extra teeth. Additionally, agenesis, the failure of one or more teeth to develop, can be a significant indicator of this anomaly. Malposition of erupted permanent teeth, where the teeth do not grow in their correct position, is another important signal that could suggest the presence of supernumerary teeth (11).

Malocclusion, or the misalignment of teeth, is another clinical sign to consider (12). This misalignment can cause aesthetic and functional problems, affecting the patient's chewing and speech. A wide interincisive diastema, which is an abnormal space between the upper front teeth, is another indicator that may suggest the presence of extra teeth. This could be caused by a supernumerary tooth preventing the teeth from coming together correctly (13).

A positive family history of hyperdontia is a relevant factor to consider. If there have been cases of hyperdontia in the family, the likelihood increases that other family members might also be affected by this condition. This suggests a possible genetic component in the etiology of the condition. Other clinical signs include the resorption of the rootsof adjacent teeth. In this process, the roots of teeth near the supernumerary teeth are gradually eroded, leading to the lossof vitality of these teeth. This can cause various problems, including sensitivity and loss of functionality of the affected teeth (14).

Finally, swelling in the vestibular or palatal/lingual areas is another sign indicating the presence of supernumerary teeth. This swelling can be caused by the impact of the extra teeth on the surrounding tissues, creating inflammation and discomfort. These clinical signs should prompt the physician to conduct further diagnostic investigations to confirm the presence of supernumerary teeth and to plan an appropriate treatment plan (15).

The treatment for hyperdontia depends on the number and location of the excess teeth and whether they are causing any pathological issues (16). Surgical intervention is often recommended to prevent malocclusion from altered normal teeth development and a thorough preoperative evaluation is necessary to minimize trauma to the surrounding hard and soft tissues (17).

CASE REPORT

A 30-year-old Caucasian patient presented with five impacted teeth (1.8, 2.8, 3.8, 4.7, and 4.8, according to the FDI World Dental Federation notation) and an impacted supernumerary tooth (distomolar 4.9). The patient experienced localized pain and slight homolateral submandibular lymphadenopathy, but no functional limitations or fever were noted. The supernumerary teeth did not cause any occlusal hindrance. Despite a lack of other stomatological pathologies, congenital anomalies, or genetic/syndromic alterations, the patient reported a hereditary link: her mother had supernumerary teeth in the posterior superior maxillary bone that had been removed. Given the hereditary aspect, it was suggested that a radiologic screening be conducted for the patient’s two sisters, aged 17 and 13, with their informed consent.

X-ray imaging revealed that both sisters also had hyperdontia and dental impaction in a systemic and non-syndromic form, with normal psychophysical development. Karyotype analysis using the G banding technique (GBT) confirmed normal male (46, XY) and female (46, XX) karyotypes for the proband, mother, and elder sister (Fig. 1).

Radiologic findings
- Elder sister: Nine impacted teeth (1.8, 1.9, 2.8, 2.9, 2.10, 3.8, 3.9, 4.8, 4.9).
- Younger sister: Four impacted teeth (1.8, 1.9, 2.8, 2.9).

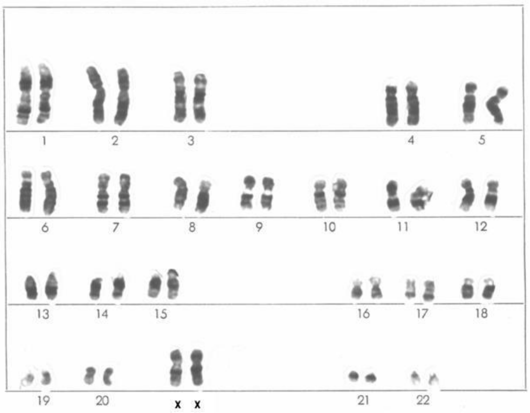

Fig. 1. *Older sister's karyotype (GBT).*

In accordance with international literature, it was decided not to extract the supernumerary teeth of the sisters, as they showed no signs or symptoms that would justify extraction. However, for the patient who initially sought attention, the decision was made to extract teeth 4.7, 4.8, and 4.9. This decision followed routine hematological investigations and assessments using X-ray dental panoramic tomography (DPT) and dental scan (DS) of the inferior maxillary bone (Fig. 2-4).

The extraction procedure successfully alleviated the patient's pain. Post-surgery, a one-week dose of intramuscular antibiotic and anti-inflammatory therapy was prescribed, including cefazolin sodium (2g/day) and ketoprofen lysine salt(200mg/day).

The clinical approach and subsequent interventions were tailored based on the absence of severe symptoms in the sisters and the presence of pain and other symptoms in the initial patient. This case underscores the importance of individualized treatment plans in managing hyperdontia, particularly when hereditary factors are involved.

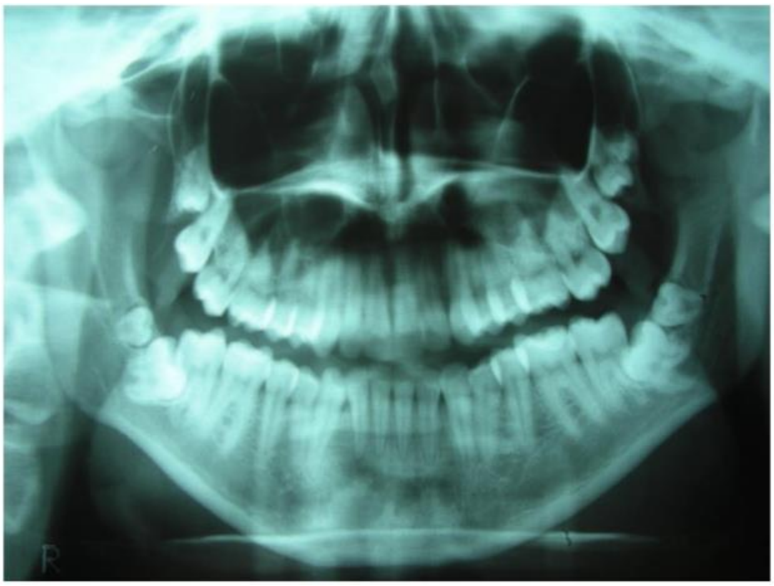

Fig. 2. *Elder sister’s X-Ray.*

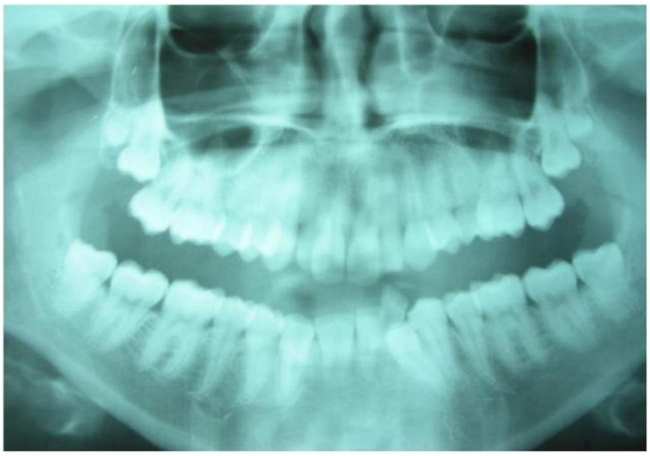

Fig. 3. *Younger sister's X-ray.*

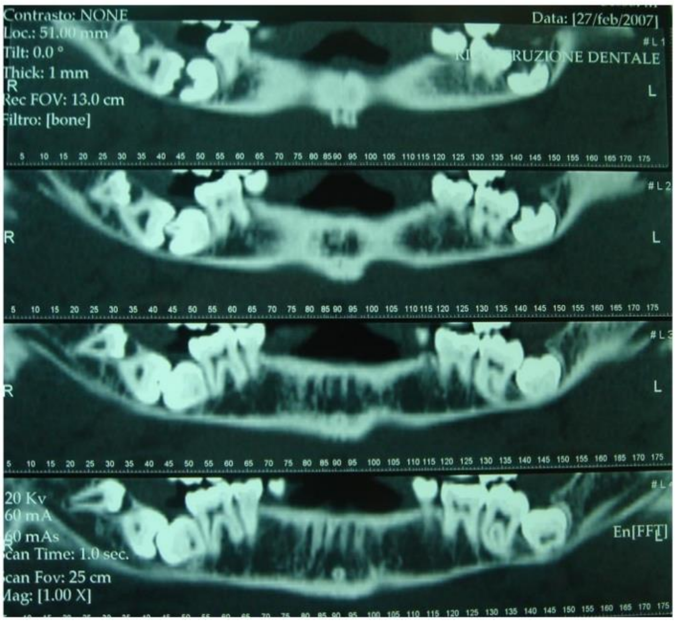

Fig. 4. *X-Ray of the mandibular arch.*

DISCUSSION

The etiology of hyperdontia remains an area of ongoing research and is not yet fully understood (18). Hyperdontia, characterized by extra teeth beyond the normal complement, can be influenced by various factors that impact odontogenesis (16). Some researchers propose that dental anomalies arise from a complex interaction of genetic factors and developmental processes (19).

One compelling theory supported by existing literature suggests that the local and independent hyperactivity ofthe dental lamina, a crucial part of early tooth development, may lead to an excessive proliferation of cells. This hyperactivity can result in the formation of additional tooth buds, ultimately causing supernumerary teeth (20).

The link between supernumerary teeth and hereditary predisposition has been extensively investigated and is widely acknowledged. Most cases of hyperdontia appear to be determined by multifactorial inheritance, which involves a combination of genetic and environmental factors. For instance, Batra and colleagues reported a nonsyndromic multiple supernumerary teeth case, which indicated an autosomal dominant pattern of inheritance (21).This means that the trait can be passed down from generation to generation, with a 50% chance of transmission if one parent carries the gene.

Supernumerary teeth are frequently associated with specific syndromes, such as cleidocranial dysplasia and Gardner's syndrome. These conditions often involve multiple systemic anomalies, with extra teeth being just one of many symptoms (22). However, it is important to note that multiple supernumerary teeth can also occur in nonsyndromic patients, though this scenario is relatively rare. This rarity underscores the importance of thorough diagnostic evaluations to differentiate between syndromic and nonsyndromic cases of hyperdontia.

A careful review of international literature, combined with the specific case described, allows us to clinically confirm the hereditary nature of nonsyndromic hyperdontia. The case highlights a rare form of hyperdontia involving bilateral, nonsyndromic multiple supernumerary teeth. The evident phenotypic penetration within the studied family unit, where multiple family members exhibit the trait—strongly suggests a genetic basis. Determining the karyotype of affected individuals can be particularly useful in excluding chromosomal pathogenesis, thus reinforcing the hereditary etiology of the condition.

CONCLUSIONS

The significance of this case report extends beyond the individual patient; it serves as a paradigm for understanding the hereditary factors predisposing individuals to hyperdontia. This understanding is crucial for effectively managing family units where this odontostomatologic anomaly is detected without syndromic forms.

In the analyzed family unit, hyperdontia in the mother and her three children, despite variations in severity, suggests an autosomal dominant trait transmission. This conclusion is supported by the observed vertical and gender-independent pattern of inheritance. The case underscores the importance of recognizing familial patterns in dental anomalies to provide accurate genetic counseling and anticipate potential occurrences in future generations. Moreover, this case confirms that surgical intervention is often necessary and effective when a constellation of symptoms accompanies the clinical manifestation of hyperdontia. Such symptoms might include pain, swelling, and dental malposition, significantly affecting a patient's quality of life. In these instances, timely and decisive surgical therapy is recommended to alleviate symptoms and prevent further complications. By removing the supernumerary teeth,clinicians can help restore normal function and aesthetics, thus improving the overall oral health and well-being of the patient.

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Conflicts of interest
The authors declare no conflicts of interest.

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Retrospective Observational Study

THE COVID-19 PANDEMIC AND THE IMPORTANCE OF EARLY RECOGNITION OF SEPSIS IN PRE-HOSPITAL EMERGENCY HEALTHCARE. A STUDY FOR FUTURE PERSPECTIVES

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ABSTRACT

In this retrospective, observational study, we evaluated all patients admitted to the 118 SET (territorial emergency system) of SG Moscati Hospital of Taranto City, Italy, between April and November 2020. Data and outcomes obtained from the 313 patients during the early phase of the COVID-19 pandemic showed that the detection of early signs of infection significantly increased the chances of surviving, especially in elderly subjects affected by pre-existing comorbidities. We found that these findings were strongly related to simultaneity and unobserved heterogeneity of conditions, perhaps also due to the poor quality of information on COVID-19 deaths. The interesting point was finding that mild and severe cases of COVID-19 showed significant differences in many aspects. The clinical signs, expression markers of blood gas analysis (ABG), complete blood count (CBC), and inflammatory patterns were evaluated in a non-intensive care setting, both in the ambulance and in the triage of the COVID-19 Unit of SET 118, and which were then used as essential markers to prevent the outcomes of COVID-19 sepsis protocol, called EESS or empirical emergency septic signs. The overall check and screening parameters based on EESS performed on a total of 313 patients gave an accurate picture that finely described the real grade of the infection with corresponding internal damages superior to RT-PCR and thoracic CT scans alone. The collected data made it possible to examine exposures to suspected outcomes, pursue predictive risk factors, and build a precise therapy to be executed starting from the ambulance, which ultimately led 118 SET to rescue the greatest majority of admitted patients, with only 17 deaths (5.4%) out of 313 patients (94.6%; male-207; female-106) in the first seven days of hospitalization.

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KEYWORDS: *Covid-19; SARS-CoV-2, Emergency Territorial System 118 (SET 118), arterial blood gas analysis (ABG), Systemic inflammatory response syndrome (SIRS), acute respiratory distress syndrome (ARDS), multiple organ dysfunction syndrome (MODS), sepsis, empirical emergency septic signs (EESS)*

INTRODUCTION

The first cases of COVID-19 in Italy were confirmed on February 21st, 2020, in a little town in Northern Italy. The Emergency Territorial System 118 (SET 118), operating in the province of Taranto City in the Apulia region, Italy, received over 5.000 calls in the period from February 2020 to November 2020, the first and the second waves of COVID-19 infection respectively (1-3). From the first cases, the peculiar characteristics of the new SARS-CoV-2 virus, the speed of contagion, and the extent of contamination immediately appeared clear, catching everyone unprepared (1). At first, the numbers could not clarify the true extent of the disease since SARS-CoV-2 was the cause of a very heterogeneous condition that often led to sudden acute respiratory failure and multiple organ dysfunction syndrome and, therefore, death (1-3).

At the beginning of the pandemic, the government regulatory body leading 118 SET activity in Puglia issued a regulation that recommended limiting ordinary residences in all but extraordinary circumstances (1). To this end, a 118 SET COVID-19 Special Unit equipped with a negative pressure system was designed and set up to deploy the immense flow of patients entering the SG Moscati hospital in Taranto in Puglia and open 24 hours a day (1). The Unit was composed of multiple interconnected sub-units, including a pre-hospital unit with bedside clinical treatment function, an emergency management team composed of doctors and nurses operating as an infection prevention and control office, and a special research team responsible for collecting data, numbers, and scientific information to support clinical management, communication coordination, laboratory, and diagnostic services (Fig.1, 2, Table I) (1).

Prehospital emergency care of suspected COVID-19 patient with acute respiratory failure

Objective

Ensure the patient classified as a suspected or proper affected case with an initial clinical picture of acute respiratory failure and/or shock the appropriate and continued emergency therapeutic support during the phases of protected transport and temporary management pending the taking charge of the dedicated hospital units.

Methodology

At home and in a mobile station (ambulance) SET-118 → acute respiratory failure → therapeutic protocol

Oxygen therapy, as needed (SpO2> 90%):

- low flow (P/F > 300 mmHg):
  with nasal goggles: 2 – 4 L/min

- high flow (P/F < 300 mmHg):
  with face mask with reservoir: 15 L/min

Non Invasive Mechanical Ventilation (SpO2< 90% or P/F < 200 mmHg + severe dyspnoea, use of accessory respiratory muscles - sternocleidomastoid, scalenes, paradoxical breathing-, RR > 35 breaths/min, pH < 7.35, pH> 7.2, Kelly 1-2)
→ CPAP: 5 – 10 cm H2O, with FiO2 of 60 – 90%

In more severe cases

In the presence of severe hypercapnia, altered mental status, hemodynamic instability, invasive mechanical ventilation IMV → ETI is indicated.

If the clinical picture compatible with bilateral interstitial pneumonia:
dexamethasone: 6 mg iv (associated with gastroprotection with pantoprazole 40 mg iv)
acetylcysteine fl300 mg iv: 2 flev in 250 ml of saline
enoxaparinafl: 1 fl4000 IU sc (in the absence of specific contraindications)

Intravenous drip with 5% glucose solution→ for nutritional purposes, in case of prolonged hospitalization.

Where an emergency vehicle with a non-medicalized but nursed crew intervenes on an unstable COVID-19 patient, the 118 Operations Center can guarantee remotely, through the CO118 doctor or even through the SET doctor specifically dedicated in service at the CO118 to carry out operations of "medical control online", real-time medical support for the administration of emergency therapy.

Fig. 1. *The admission to the pre-hospital unit was created to avoid overloading the hospital in the Taranto areas. The admission phases were based on each patient's clinical condition at acceptance into the 118 COVID-19 Unit. The therapies could be delivered at home or performed on-site based on symptoms and ABG parameters.*

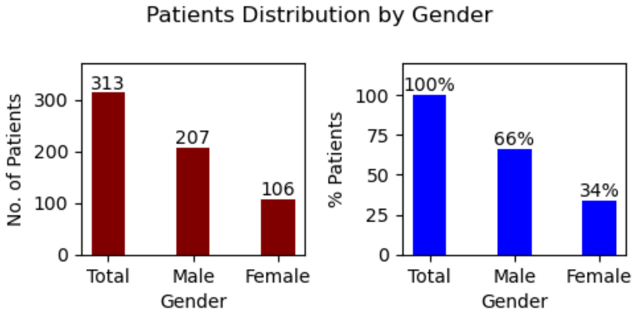

Fig. 2. *Male prevalence in COVID-19 infection. The red bars reflect male predominance, 207 vs 106 females. The data was obtained from SET 118 of Taranto at the S. Giuseppe Moscati hospital in Taranto.*

Table I. *EESS scores recorded at the time of 118 SET ambulance and 118 SET triage*.

Vital signs	**SCORES**		
	Light	**Medium**	**Severe**
Age	<30 YO 1	31-55 YO 2	>56 YO 3
Alert and conscious	1	2	3
Body temperature Hypothermia or fever	1	2	3
Muscle ache	1	2	3
Dyspnea	1	2	3
Dry cough	1	2	3
Nausea and vomiting	1	2	3
Headache	1	2	3
Fatigue	1	2	3
Anosmia	1	1	1
Ageusia	1	1	1
Diarrhea	1	2	3
Skin rashes	1	2	3
BP	1	2	3
Rr	1	2	3
Hr	1	2	3
Time from 1st symptom	<3 days 1	Between 4-5 days 2	>5 days 3
Tot score	17	32	47

In 2 out of 5 cases that became symptomatic, the condition worsened within a few hours, the initial symptoms always starting from a feverish state followed by dry cough, ageusia, anosmia, muscle pain, conjunctivitis, and headache, which tended to quickly lead to severe dyspnoea, coma and finally death (4-6). It soon became clear that the septic risk increased dramatically starting from the 3rd/4th day and became almost irreversible from the fifth day onwards. Critically ill patients affected by COVID-19 began to develop respiratory failure within a few hours of the onset of the first symptoms. Therefore, the immediate identification of precursor signs was considered extremely important (4-6). Starting from day 4/5, a gradual worsening of the state of alertness could be observed with an increase in confusion, although without clear signs of respiratory distress, which usually appeared suddenly and was later called "happy hypoxemia" or "silent hypoxemia". In all these cases, dyspnea with marked arterial hypoxemia and hypocapnia was proven with further investigation through blood gas analysis (ABG) (Fig. 3-5) (7, 8).

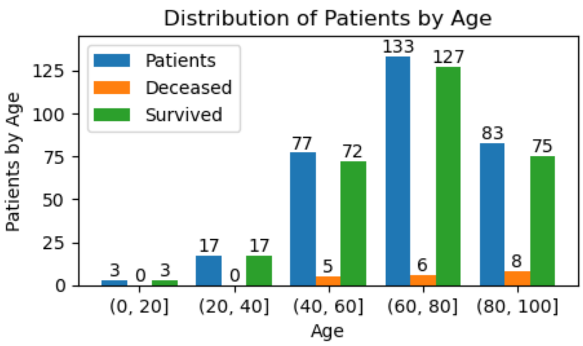

Fig. 3. *In Italy, fewer young people are known to have had the disease. As with older people in other European countries, our findings confirmed that the most affected were old and male between the ages of 60 and 80.*

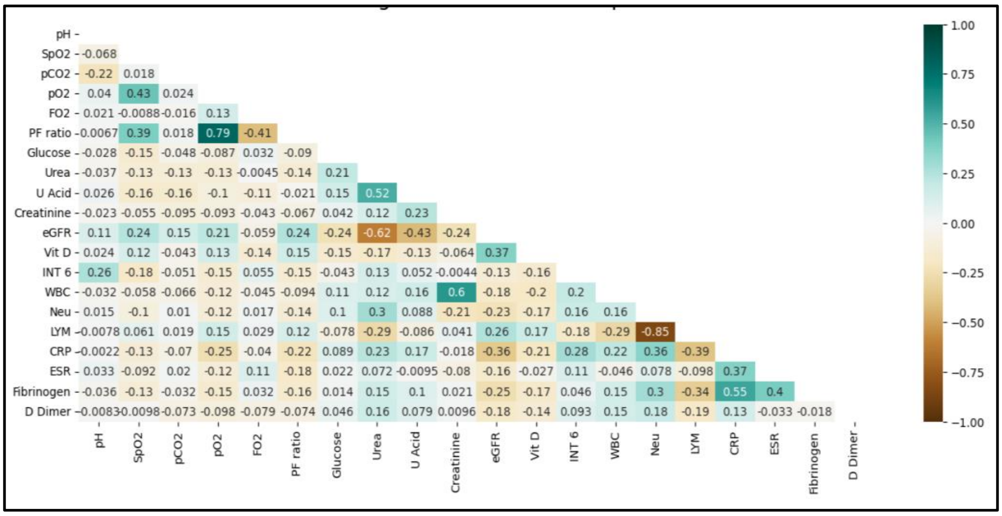

Fig. 4. *The triangle correlation heatmap shows correlations between different blood parameters for the entire group consisting of 313 patients. Correlations were considered positive and negative on different parameters of blood, ABG, and inflammatory markers. Positive, strong significance was seen between PF and pO_2 (+0.79, P-value > 0.05), WBC and creatinine (+0.6, P-value > 0.05), fibrinogen and CRP (+0.55, P-value > 0.05), uric acid and urea (+0.52, P-value > 0.05); strong negative significance was observed between lymphocytes and neutrophils (-0.85, P-value > 0.05), eGFR and urea (-0.62, P-value > 0.05); medium negative significance was observed between eGFR and FO_2 (-0.41, P-value > 0.05), between PF and FO_2 (-0.41, P-value > 0.05).*

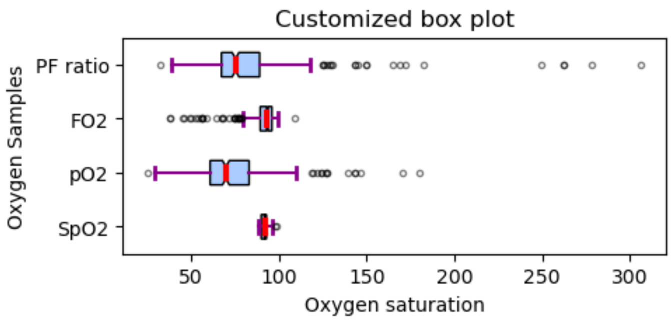

Fig. 5. *Box plot comparing oxygen saturation medians by SpO_2, pO_2, FO_2, and PF ratio value and by outcome. The PF ratio shown in the figure above has a mean value of 82.5 with a standard deviation (SD) of 32.5. The distribution shows an extremely broad upward range of values, and few patients have extremely high values in the PF ratio. The FO_2 shown in the figure above has a mean value of 90.3 with an SD of 10.4. The values of FO_2 have a solid distribution but have quite a few downward extreme outliers. The pO_2 shown in the figure above has a mean value of 73.1 with an SD of 21.3. The values of FO_2 have quite a wide distribution and a reasonably high number of upward extreme outliers. The SpO_2 shown in the figure above has a mean value of 91.5 with an SD of 1.9. The values of SpO_2 have quite a narrow distribution with practically no extreme outliers.*

This required an epochal turning point in the attitude of emergency personnel and the activation of an important information campaign to avoid the intensification of contagion in the logic of "Intervene at the first symptoms" (7-10).

The most relevant comorbidity traits of COVID-19 patients were aging (70 to 90 years), cardiovascular disease, type 2 diabetes mellitus, chronic kidney condition, and chronic neurological disease (7, 11). The transport of critical patients has represented one of the most delicate and vulnerable aspects in the management of COVID-19 emergencies, both in city areas and in the most isolated territories (12-15).

The etiopathogenesis was related to deep and severe endothelitis, considered the main factor of decay of the microcirculatory mechanism. A picture closely connected to a generalized increase in uncontrolled inflammatory processes, known as a "cytokine storm" which led to microvascular thrombosis followed by a rapid and silent necrotic process that ended in coma and often in death (15, 16). The pathophysiology of severe COVID-19 resembles typical aspects of septic disease characterized by a progressive worsening of vital functions and composed of three main phases: the systemic inflammatory response syndrome (SIRS), the acute respiratory distress syndrome (ARDS), which concludes in multiple organ dysfunction syndrome (MODS) and rapid general decline of the body with death (15, 16). The CT procedure showed in detail what was happening in the infected lungs. Images revealed established atelectasis, suggesting bilateral interstitial pneumonia characterized by ground-glass opacity images (17-19).

EESS procedure and the limitations of current predicting screenings for COVID-19 sepsis

To this end, we created an early empirical emergency protocol called EESS (early empirical septic signs), which was started in the ambulance and completed in triage, allowing us to predict the progression of the individual disease patient with a high degree of precision. Unlike normal operations, 118 Unit crews were used outside of routine dispatch procedures. They were autonomous in selecting COVID-19 patients for care based on EESS parameters to identify high-acuity patients who likely required first aid rescue, aerosolization, and medical interventions in ambulance and triage (1). Crews received SMS alerts from ambulances for calls involving “clear, immediate life-threatening” criteria, including blood gas parameters, altered level of consciousness, breathing difficulty, rash, gastrointestinal distress, urogenital distress, dry cough, fever, heart disease, arrest, blood count (in triage) and existing comorbidities among others (1). Each symptom and sign has been assigned a score; the higher the score, the greater the possibility of septic risk related to COVID-19 (Table I).

Analyzing similar data from the literature, our results were consistent with data from other studies on CRP, IL-6, lymphopenia, and neutrophilia (10, 11), which tended to be associated with an unfavorable outcome of the illness. Until that moment, however, the greater reliability of the PaO2/FiO2 ratio compared to COVID-19 infection and other inflammatory tests in predicting sepsis had not yet been highlighted (20, 21)

Sepsis is a condition characterized by a chain of events triggered by an infection, which can be bacterial, viral, fungal, or protozoan. The initial stage of the infection can begin anywhere in the body: intestine, stomach, brain, bladder, kidneys, chest, or even skin. Following abnormal biochemical changes, the body releases chemicals, proteins, and immune mediators into the bloodstream in an attempt to fight the infection (22-24).

If left unchecked, these immune mediators trigger widespread inflammation, blood clots, and leaky blood vessels. Once blood flow is compromised, organs are left without sufficient nutrients and oxygen, causing tissue damage (23-25). The burden of sepsis on ambulance services remains an issue to be improved; data from previous studies suggested that 7% of emergency cases in first aid deal with patients with infections, of which around 8-10% have an infection that will be diagnosed as sepsis (5, 6, 24).

Several sepsis-related screening modalities have been developed for the early evaluation of patients during ambulance care. Each measure is based on certain scores that are related to the presence of two or more symptoms, such as the PREhospital Severe Sepsis (PRESS) score, the Systemic Inflammatory Response Syndrome (SIRS), the Robson screening tool, the Sepsis Alert Protocol, Quick Sequential (sepsis-related) Organ Failure Assessment (q-SOFA) and BAS (based on oxygen saturation, respiratory rate and systolic blood pressure) (25-28).

PREhospital Severe Sepsis PRESS

The PRESS screening tool has a sensitivity score of 86% and a specificity score of 47%. This evaluation and screening procedure was constructed by combining data from over 66,000 urgent and emergency transports, allowing us to detect entry criteria based on the "severe sepsis" diagnosis. Using univariable logistic regression analysis, the researchers proposed typical clues and criteria of sepsis: 1) temperature, 2) systolic blood pressure, 3) heart rate, 4) respiratory rate, 5) oxygen saturation, 6) glucose and 7) Glasgow Coma Scale.

Subsequent additions were made: (1) a heart rate greater than 90 beats per minute, (2) a respiratory rate greater than 20 beats per minute, and (3) a systolic blood pressure less than 110 mm/Hg. However, the PRESS scoring system has the limitation of being an internal validation tool, and therefore, further research is needed to validate the external validity of the results (28-31).

Systemic inflammatory response syndrome (SIRS)

Although SIRS describes physiological signs indicative of the transition from infection to sepsis, it lacks indicators specific to sepsis. In fact, SIRS produces a wide variety of insults other than infection, which results in sepsis.

Further evaluations then demonstrated that the SIRS criteria in the department did not prove to be reliable predictors of sepsis or mortality. It is concluded that the use of SIRS criteria to identify sepsis in an emergency and pre-hospital environment could, therefore, be equally ineffective since body temperature >38°C or <36°C, heart rate >90 beats/min, respiratory rate >20 breaths/min and white blood cell counts >12,000/nm3 or >10% immature neutrophils are general concepts and not specific for infection (31, 32).

Sequential organ failure assessment (SOFA)

By consensus, the experts proposed a new definition of sepsis as life-threatening organ dysfunction, defined by a SOFA (sequential organ failure assessment) score ≥ 2. Septic shock was defined as the need for a vasopressor to maintain a mean arterial pressure of at least 65 mmHg and a serum lactate level greater than 2 mmol/L (>18 mg/dL) in the absence of hypovolemia that may increase the rate mortality rate at 40% (33, 34).

Rapid quick-SOFA (q-SOFA)

The q-SOFA score was introduced to provide further precision to circumvent these limitations and predict the risk of death and prolonged stay in intensive care. However, q-SOFA was not designed to stand alone as the sole method of early warning reporting of sepsis or for identifying which patients should be transferred to the ICU or elsewhere, always wearing protective equipment (35-37).

BAS (based on oxygen saturation, respiratory rate, and systolic blood pressure)

BAS indicators 90-30-90. This procedure is based on three variables, and patients are considered septic if one or more of the three clinical indicators result in a positive. Therefore, the oxygen saturation level is less than 90%, the respiratory rate is greater than 30 breaths per minute, and the systolic blood pressure is less than 90 (35, 38).

The limits of these procedures are given by the need for some laboratory tests that absolutely cannot be performed in the restricted context of the ambulance, as well as in the triage area of an emergency room.

Additionally, the time required to obtain test results can cause a significant delay in detecting a septic patient. A further problem is the low quality of sepsis prediction and screening protocols in the pre-hospital setting (low precision and often imprecise) (38).

MATERIALS AND METHODS

All patients included in this study were handled by 118 ambulances and admitted into SET 118 emergency facility of SG Moscati Hospital of Taranto City. Patients were classified according to the Italian Triage and EESS. The EESS was determined using six judgment metrics: age, sex, physical signs, hemodynamics, metabolic, and ABG analysis. Overall, symptoms such as alertness/responsiveness, vomiting, diarrhea, skin color, presence of rushes, muscle ache, cough, breathing difficulties, ageusia, anosmia, headache, systolic/diastolic blood pressure (BP), heart rate (HR), respiratory rate (RR), body temperature (hypothermia and fever), arterial blood gas analysis (ABG) for oxygen saturation, fraction of inspired oxygen (FiO_2, SpO_2, pO_2, pCO_2, PF ratio, and pH) were all assessed in the ambulance and given a score between 32 to 47 high risk and 31 to 17 low risk (Table I). We also obtained clinical data, including patient history, comorbidities, and time of onset of first symptoms.

Patients with medium and high scores were suspected of sepsis and received appropriate treatment in accordance with the recommendations of the 1-hour sepsis bundle. Empirical broad-spectrum antibiotics and antihistamines were administered within 1 hour, and fluid resuscitation and vasopressors were implemented if necessary (38).

Study population

The laboratory panel and clinical/radiological data were obtained after signing the informed consent by all patients recruited in the present investigation, in accordance with the Declaration of Helsinki. The study was approved by the Internal Committee of SG Moscati Hospital of Taranto. The subjects were male and female, aged 21 to 95 (n = 313). All patients underwent a screening process for COVID-19 at the 118 Emergency and Pre-Hospital Department of SG Moscati Hospital of Taranto City between September and November 2020. All patients were routinely tested for COVID-19 using nasopharyngeal swabs assessed by RT-PCR and thoracic CT scans. The diagnosis of COVID-19 was retrospectively evaluated.

The pulmonary and renal systems were the most common site of infection (85%), followed by the cardiovascular system (15%). The cohort was then divided into two groups and were compared: 17 deceased (5.4%) patients (score <31) and 296 (94.6%) patients were placed in a group of survivors (score 32-47). A flow diagram illustrates patient enrollment and group allocation (Table I).

RT-PCR and nucleic acid detection

The COVID-19 kit (Real-Amp kit) was provided by OSANG-Healthcare of Korea Co. Ltd. (Seoul, Korea). The COVID-19 nucleic acid test detects the N and 1ab genes, and the quality of the nucleic acid extraction was monitored using an internal standard. For each set, a reaction mix, a probe mix, a positive control, and a negative quality control were used. The result was considered positive when two genes were amplified, and the cycle threshold value was detected below the detection limit. To reduce variability and bias, the entire sample preparation, nucleic acid extraction, and dilutions were performed in a different laboratory in the same city at the SS Maria Annunziata Hospital in Taranto (Puglia, Italy). Viral RNA was extracted using the GeneFinder™ COVID-19 PLUS RealAmp Viral RNA Kit (OSANG Healthcare Co., Ltd., Anyang, Korea), according to the manufacturer's instructions from nasopharyngeal swab and sputum samples. The COVID-19 PLUS Reaction Mix and 5 μL of COVID-19 PLUS Probe Mix were used to prepare the RT-PCR Master Mix, following the manufacturer's instructions for each set. A sufficient amount of master mix was prepared for all reactions plus extra amounts to avoid possible pipetting errors (total number of master mix = n sample + 1 positive control + 1 negative control + 1 extra). Next, 15 μL of RT-PCR Master Mix was poured into each PCR tube or 96-well plate, 5 μL of sample RNA was added into the corresponding PCR tube/well plate for amplification, and each well was mixed pipetting. 5 μL of positive control and negative control were placed in a single PCR tube and placed for testing in the real-time thermal cycler for amplification.

Statistical analysis

Statistical significance was determined using Student's t-test with 95% confidence comparisons between the four groups. Where indicated, the z score of the percentage of marker expression was calculated as follows: $z = (x - \mu)\sigma$, where x is the raw score, μ is the mean of sample distribution, and σ is the SD (95%). Fisher's exact test was used for categorical comparisons. Significant PhenoGraph clusters ($p \leq 0.05$) were determined by chi-squared goodness-of-fit tests comparing the relative abundance of each categorical group in each PhenoGraph cluster relative to input. A standard model for describing the evolution of the infected cases by viruses can be constructed as follows:

$dxdt=E(x)*I(x), with\ E(x)=\lambda*x,\ I(x)=1-x\^b$

where x(t)=N(t)/Nmax, x0=N0/Nmax; N(t) is the number of total infected cases evolved from the initial N0=N(0) cases, Nmax is the maximum possible number of infected cases; λ is the exponential growth rate and becomes clear for x(t) << 1 where I is negligible, leading to:

$x=x\ (0)*\exp(\lambda t)$or $N(t)=N\ (0)*\exp(\lambda t)$, for $x\ll1$ where $I\ (x)$~1

The function of negative feedback, I, models the factors that flatten the curve, such as the measures taken against spreading. While these factors are not affecting the exponential growth rate λ, they become more effective as the number of cases increases, getting closer to Nmax; exponent b controls the effectiveness of these factors; strict measures correspond to smaller values of b.

RESULTS

Validation cohort

To validate COVID-19 risk biomarkers for identifying patients who later developed critical illness, we retrospectively analyzed the clinical and biochemical data of 313 patients collected. A group of expert doctors collected and verified the variables required to validate the disease.

Observations

The blood test component indicates the name of the column or parameter for which the t-test was performed; the T-statistic measures the difference between the means of two groups. For instance, in the case of pO_2, the T-statistic was -5.194828; the *P-value* is the probability of observing a T-statistic as extreme as the one computed, assuming that the null hypothesis is true (i.e., assuming that there is no significant difference between the two groups), in the case of pO_2, the *P-Value* was 0.000055; significance provides a summary of the statistical significance based on the *P-Value*. If the *P-value* resulted in less than a chosen significance level (alpha set to 0.05), it was considered statistically significant. In this case, with a P-value of 0.000055, the result is labeled as "Significant," suggesting enough evidence to reject the null hypothesis. Therefore, such could be the case of the interpretation of pO_2; the T-statistic was -5.194828, and the associated *P-value* was 0.000055. Since the *P-value* is less than the typical significance level of 0.05, the null hypothesis has to be rejected. This means that, based on the available data, there was enough evidence to suggest a statistically significant difference in the pO_2 between the two groups being compared.

Primary outcomes

Firstly, we wondered whether investigating vital signs, ABG, and symptoms such as BP, RR, HR, consciousness and alertness, fever, GI tract, and urinary tract manifestations was a reliable tool for classifying patients infected with SARS-CoV-2 and predicting the outcome (1, 3, 39). Distribution by age and sex indicated that older adults, male, aged 60 to 80 years old, same as in other European countries, were confirmed to be the most affected (Fig. 1). Distribution of the clinical course of COVID-19 pneumonia and parameters related to BP, RR, HR, consciousness and alertness, fever, GI tract, and urinary tract showed that both survivors and deceased have not significant differences since both revealed slightly high BP (diastolic and systolic), fast RR, slight fever, dry cough conscious or semi-alert at the admission (1, 3, 39).

Secondary outcome

We were then interested in comparing the value of the PaO2/FiO2 ratio with inflammatory markers, different functions (CRP, IL-6, Fibrinogen, and eGFR), and CBC (neutrophils, lymphocytes), already known to be useful in predicting the outcome of patients with SARS-CoV-2 infection (1, 3, 37). The correlation coefficient were considered either positive or negative. In the general population (313 patients), the triangle correlation heatmap showed important correlations between different parameters. The results revealed a strong negative correlation with neutrophils and lymphocytes (-0.85), a medium-strong negative correlation with eGFR and urea (-0.62); it has been observed medium negative correlation coefficient with eGFR and uric acid (-0.43); it has been observed medium low negative correlation coefficient with PF ratio and FO_2 (-0.41) (P-value > 0.05). Conversely, there was a strong positive correlation coefficient between PF ratio and pO_2 (+0.79); it has been observed that there was a strong positive correlation coefficient with WBC and creatinine (+0.6), both markers were changing in the same direction, i.e., high WBC-ratio was accompanied with high creatinine level, and low WBC-ratio was accompanied low creatinine level (P-value > 0.05); it has been observed strong positive correlation coefficient with fibrinogen and CRP (+0.55) (P-value > 0.05); it has been observed medium positive correlation coefficient with pO_2 and SpO_2 (P-value > 0.05). There was a high correlation coefficient between PF-ratio and pCO_2 (+0.79), and values of PF-ratio and pCO_2 were changing in the same direction, i.e., high PF-ratio was accompanied by high pCO_2 and low PF-ratio value was accompanied low pCO_2 (P-value > 0.05).

Survivors and deceased, the correct comparison

The deceased group demonstrated a significantly lower median/mean for admission for age (deceased-73.88 vs survivors 67.7); time from the first symptoms before SET 118 admission (>4 days for deceased vs <4 days survivors) (P-value > 0.05); ABG (%) PF ratio (deceased-58.63 vs 83.92 survivors) (p-value > 0.05), pO_2 (deceased-51.15 vs 74.41 survivors) (p-value > 0.05); diastolic BP (mmHg) 75.06 (12.87 total 313, Mean SD), survivors (76.50 vs deceased 70.60 Mean SD) (p-value > 0.006); systolic 126 (113–140 total 313 patients median IQR), survivors 124 (112–134) vs deceased 130 (107.5–143) (median IQR) (P-value > 0.495); RR 21 (20–24 total on 313), 20 (20–23 survivors vs 24 20–28 median

IQR), survivors (125/114–136 vs deceased 30/107.5–143 median IQR) (P-value > 0.05); CRP (deceased-126.2 vs 72.31-survived) (p-value > 0.05); Uric acid (deceased-6.8 vs 5,72-survived) (P-value > 0.05) were the main markers statistically significant between who eventually survived and who deceased (p-value > 0.05). Age, oxygen saturation, respiratory rate, systolic-diastolic, and BP were associated with a course of infection on univariate analysis. Of note, there were differences between survivors and deceased mainly related to WBC (neutrophilia vs lymphopenia), creatinine, glucose, IL-6, CRP, and fibrinogen levels (Fig 6-15; Table II).

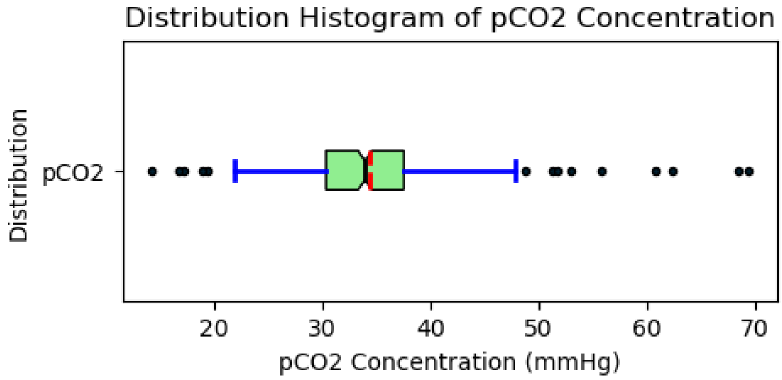

Fig. 6. *pCO_2, shown in the figure above, has a mean value of 34.3 with an SD of 6.7. The values of pCO_2 have quite a wide distribution and a high number of extreme outliers in both directions. While this may represent a large range of values in real life, it might be interpreted as a typical feature of COVID-19 infection.*

Table II. *Data from two groups of patients, deceased and survivors, with statistical significance levels for each considered marker.*

Comparison Deceased Survivors	**Blood_Test_Component**	**T-Statistic**	**P-Value**	**Significance**
4	PF ratio	-5.702471	0.000007	Significant
2	pO_2	-5.194828	0.000055	Significant
16	CRP	2.299997	0.034367	Significant
7	U Acid	1.744001	0.098284	Significant
10	eGFR	-1.558498	0.13651	Not Significant
5	Glucose	1.472471	0.158983	Not Significant
15	LYM	-1.395428	0.179597	Not Significant
0	SpO_2	-1.354904	0.192394	Not Significant
18	Fibrinogen	1.345257	0.194576	Not Significant
12	INT 6	1.299453	0.211799	Not Significant
3	FO_2	-1.231342	0.23472	Not Significant
13	WBC	1.227227	0.237317	Not Significant
6	Urea	1.147982	0.26429	Not Significant
8	Creatinine	0.971729	0.345636	Not Significant
19	D Dimer	-0.928007	0.359733	Not Significant
1	pCO_2	-0.671245	0.511259	Not Significant
9	pH	0.613789	0.546125	Not Significant
11	Vit D	0.477367	0.639421	Not Significant
14	Neu	0.24968	0.805867	Not Significant
17	ESR	0.141681	0.888985	Not Significant

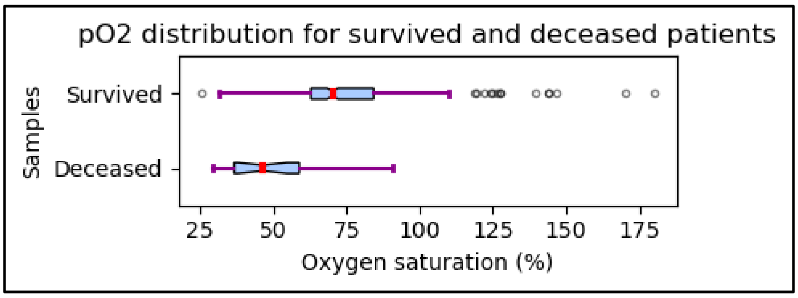

Fig. 7. *Clinical course of COVID-19 pneumonia of pO_2 ratio distribution between survivors and deceased. Box plots (pO_2 distribution from Min. to Max. in relation to oxygen saturation %).*

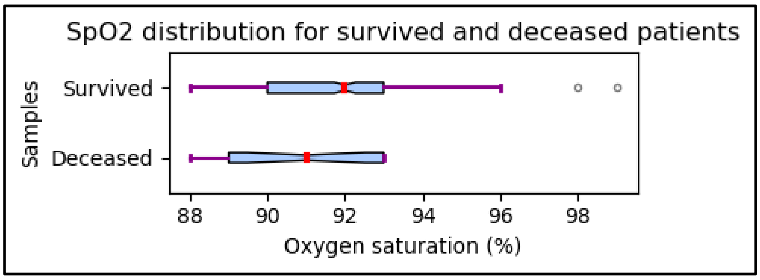

Fig. 8. *Clinical course of COVID-19 pneumonia of SpO_2 distribution between survivors and deceased. Box plots (SpO_2 distribution from Min. to Max. in relation to oxygen saturation %).*

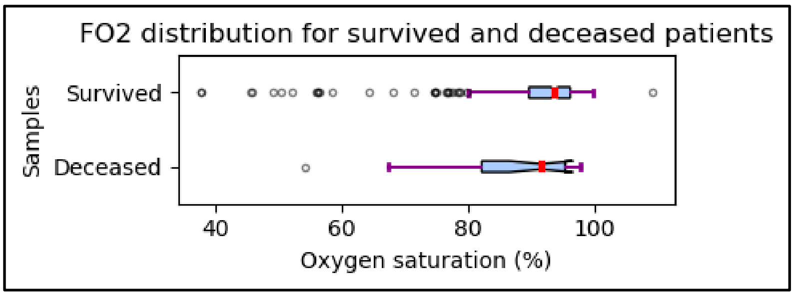

Fig. 9. *Clinical course of COVID-19 pneumonia of FO_2 distribution between survivors and deceased. Box plots (FO_2 distribution from Min. to Max. in relation to oxygen saturation %).*

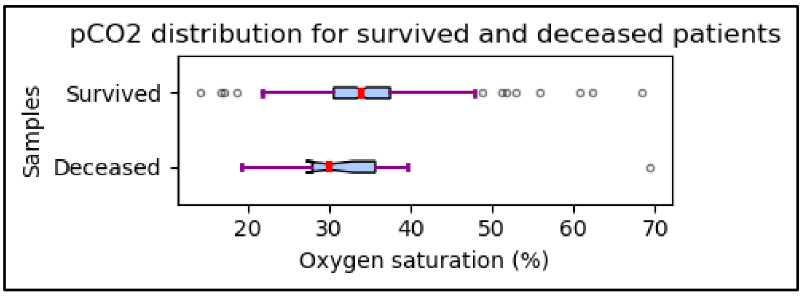

Fig. 10. *Clinical course of COVID-19 pneumonia of pCO_2 distribution between survivors and deceased. Box plots (pCO_2 distribution from Min. to Max. in relation to oxygen saturation %).*

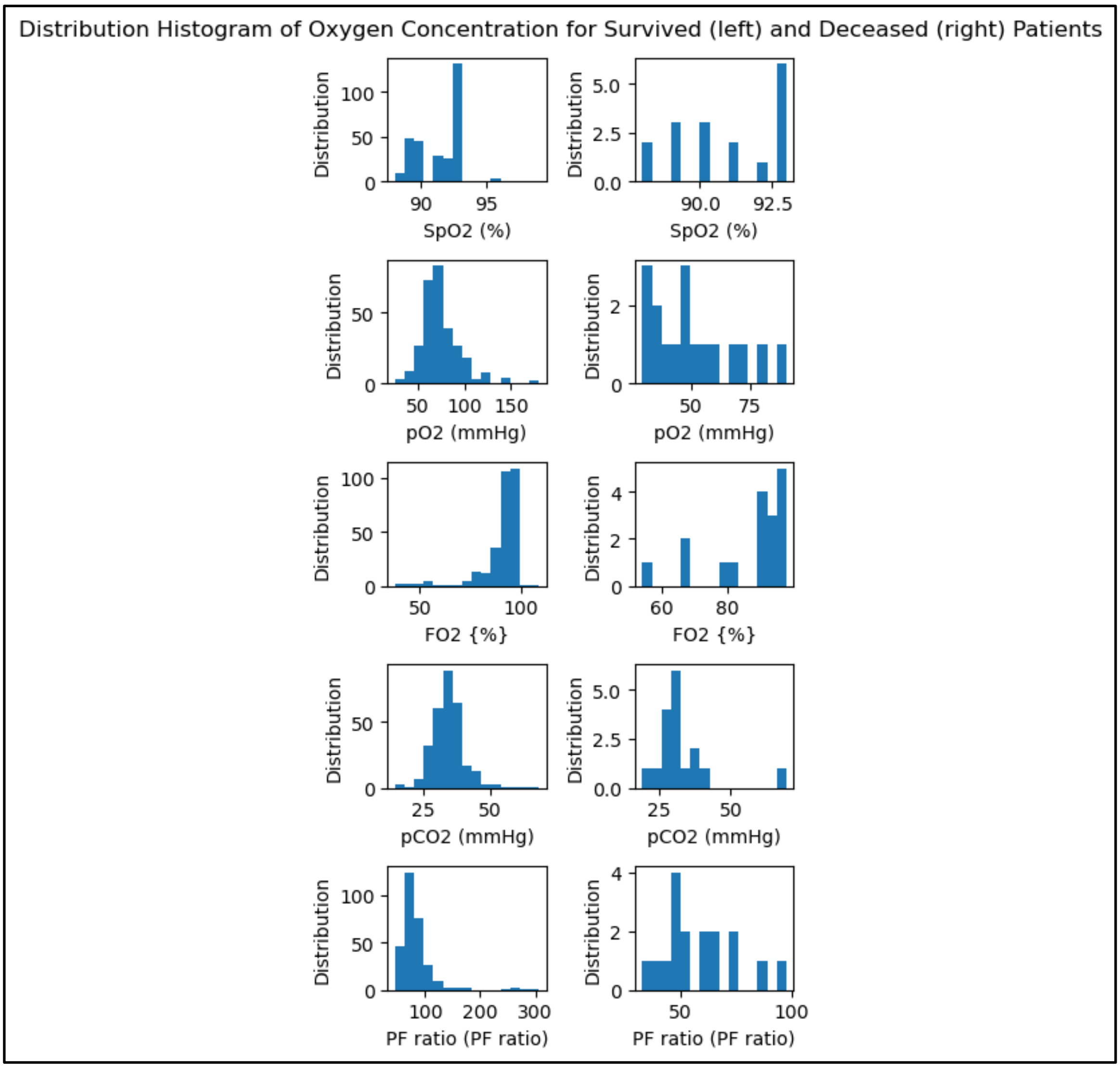

Fig. 11. *Histogram showing the distribution of the clinical course of COVID-19 pneumonia of main ABG markers between survivors and deceased.*

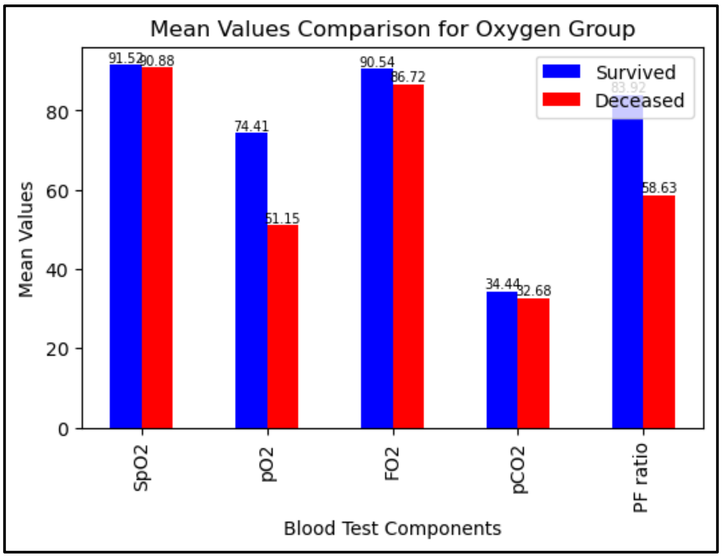

Fig. 12. *Histogram showing the distribution of the clinical course of COVID-19 pneumonia of main ABG markers between survivors and deceased. PO_2 and PF ratio are confirmed as the main variable between survivors and the deceased.*

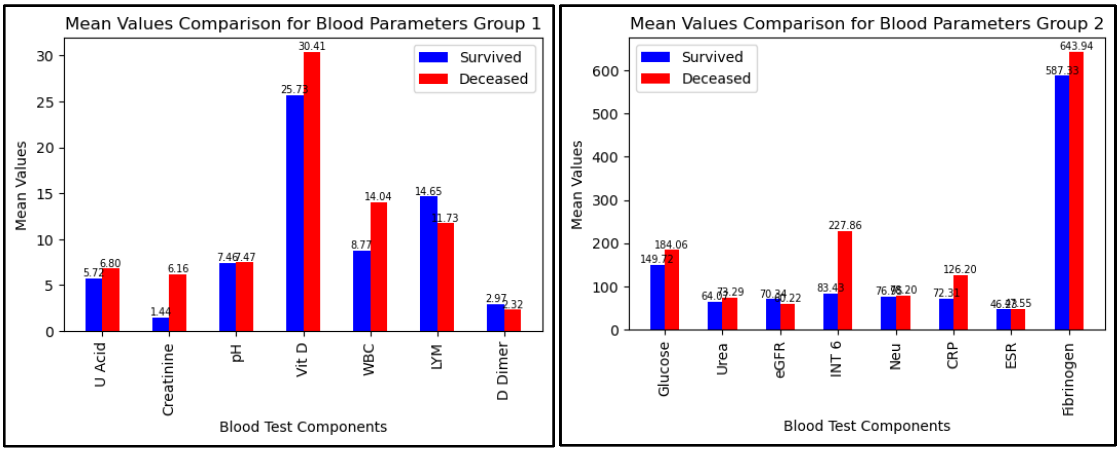

Fig. 13. *Histogram showing the distribution of the clinical course of COVID-19 pneumonia of main blood parameters between survivors and deceased. Though non-significant, there were differences between survivors and deceased mainly related to WBC (neutrophilia vs lymphopenia), creatinine, IL-6, CRP, and fibrinogen levels.*

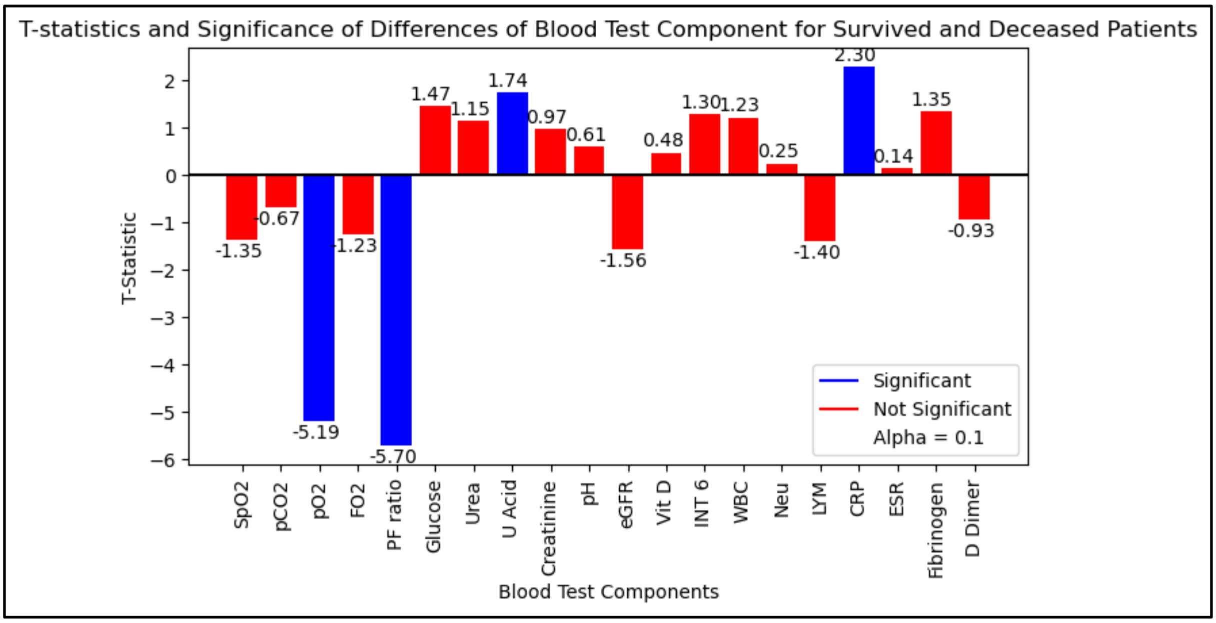

Fig. 14. *Histogram showing the distribution of the clinical course of COVID-19 pneumonia of main blood parameters between survivors and deceased. Strong significant differences were observed between survivors and deceased, mainly related to pO_2, PF ratio, urea, and CRP levels.*

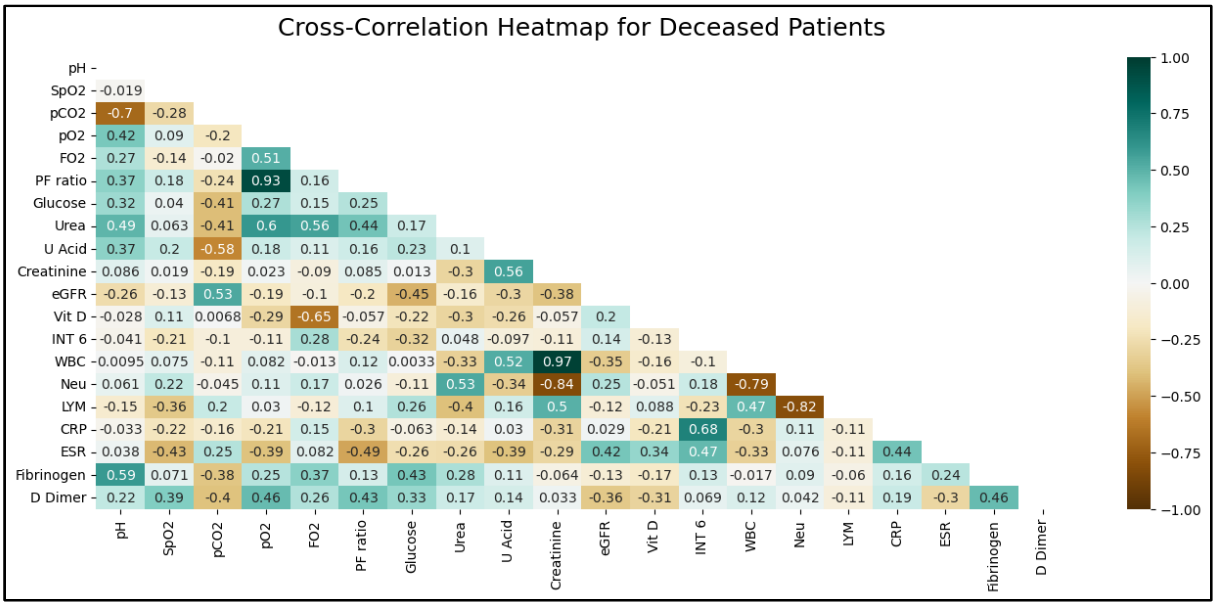

Fig. 15. *The triangle correlation heatmap shows correlations between different parameters for the entire group of deceased patients. The correlation was considered positive and negative on different parameters of blood, ABG, and inflammatory markers. Positive strong significant correlation were seen between PF and* pO_2 *(+0.93, p-value > 0.05), WBC and creatinine (+0.97, p-value > 0.05), IL-6 and CRP (+0.68, P-value > 0.05),* pO_2 *and urea (+0.6, p-value > 0.05); significant negative correlation was observed between creatinine and neutrophils (-0.84, p-value > 0.05), neutrophils and lymphocytes (-0.82, p-value > 0.05); strong negative significance was observed between neutrophils and WBC (-0.79, p-value > 0.05), strong negative significance was observed between* pCO_2 *and pH (-0.70, p-value > 0.05) significantly medium-strong correlation between vitamin D and* FO_2 *(-0.41, p-value > 0.05).*

DISCUSSION

During COVID-19, age, vital signs, level of alertness, blood gas analysis values , and comorbidities were the first parameters to be assessed, starting with ambulance assistance. Pre-existing problems such as kidney or lung disease were associated with increased susceptibility to COVID-19 sepsis (12, 13). Elderly males were the most affected as they showed a higher prevalence of chronic comorbidities such as diabetes, heart disease, and respiratory problems. With age, the immune system undergoes age-related changes that necessarily facilitate the onset of functional damage in both cell-mediated and humoral immunity (12). The possible contribution of this work to the literature consisted in highlighting in our cohort of patients, deceased and survivors, among the general markers and variables analyzed, our EESS score table as a useful independent prognostic biomarker to predict the first signs of progression of sepsis in COVID-19 patients.

The degree of accuracy of the EESS protocol allowed us to reduce the mortality rate during the first 7 days of hospitalization, with only 17 deaths out of 313 patients hospitalized in the SET 118 COVID-19 Special Unit. In line with other procedures, patients with suspected sepsis were managed by implementing resuscitation procedures to which were added innovative therapeutic practices not yet present in the clinical procedure of the healthcare system, such as double antibiotic therapy and N-acetyl cysteine (38-41). All patients received intravenous fluid resuscitation with an initial intravenous fluid bolus of 30 ml/kg, targeted fluid resuscitation, and oxygen (CPAP mask); all received empiric antibiotics, corticosteroid, and antioxidant therapy adapted to the presumed degree of infection and were referred for early therapy and control procedures (41-43). Particular attention was given to patients with right heart failure, acute kidney injury (AKI), febrile and semi-alert, or those with respiratory failure related to hydrostatic-pulmonary-edema disease (42-44).

Furthermore, the present study found that the systemic inflammatory response and secondary organ damage caused by COVID-19 were due to a complex combination of several factors and co-variables to be considered the innermost causes of "happy hypoxemia". During the COVID-19 pandemic, the scarcity of studies and data was the main cause of inadequate therapies and beliefs and, therefore, of a large number of failures; secondly, national guidelines have focused exclusively on hospital recognition, giving little importance to the pre-hospital phase of the emergency (44, 45). Nowadays, guidelines are all in favor of starting treatment within one hour of the first signs and symptoms of suspected sepsis, which can only be achieved if sepsis becomes a priority in home/hospital care, emergency, and ambulance (46).

We observed that the irreversible inflammatory patterns that eventually led to sepsis were also linked to secondary spread into local alveolar interstices as a consequence of some bacteria invasion into the lungs together with Sars-CoV-2 virus (*S. Aureus, E. Coli, A. baumannii, Enterococcus spp., K. pneumoniae, etc.*) (46-49). In these patients, the steady progression of the infection from local to systemic generated a generalized inflammatory state characterized by a dysregulated immune response that silently progressed to massive secondary damage to organs and tissues (46-49).

Bacteria cardiolipin, prothrombin, albumin and platelet aggregation factors, and prothrombin antibodies were found in nearly half of patients with COVID-19 (50-52). Even more, it was confirmed that anti-cardiolipin antibodies were observed to be involved in the increase in neutrophils with a consequent increase in extracellular traps (NETs), associated with an increase in clotting speed, a condition found in almost 100% of the lungs of severely affected COVID-19 patients (CT -scan of ground glass opacities) (49, 50).

From infection to ARDS to MODs, clinical features of COVID-19 sepsis, general correlation of the whole 313 group

We were also interested in comparing multiple vital sign values , including alertness and responsiveness, fever, RR, BP, HR, ECG, and ABG analysis. Such multiparametric measurements of the *in vivo* microenvironment provided unique insights into biological processes in severely affected COVID-19 patients and their response to treatment (1, 4, 7, 48).

Abnormalities in vital signs and blood gas levels are considered highly suggestive of a silent progression of sepsis. At the same time, inflammatory parameters represented by fibrinogen, glucose, IL-6, and CRP had a strong-moderate predictive value among COVID-19 survivors and deceased at 1 week, highlighting clinical differences between severely infected individuals from those with mild disease with a non-septic course (Fig. 8) (46, 47, 49).

Blood gas values have been widely used to describe the progression of pneumonia towards ARDS and MODS (47-51). However, we focused on the PF ratio because of its strength in emphasizing the precise dynamism of microcirculatory exchange rather than static criteria (Table II). According to some authors, a moderate to severe impairment of the PF ratio was independently associated with a three-fold increase in the risk of in-hospital mortality, concluding that the severity of respiratory failure is useful for identifying patients at the highest mortality risk (52). A PF ratio ≥ 300 has been considered strongly indicative of acute respiratory failure and has, therefore, been associated with significantly unfavorable outcomes in patients with COVID-19 (50).

Factors contributing to SARS-CoV-2 pathophysiology

SARS-CoV-2 is transmitted through respiratory droplets and aerosols, with an average incubation period of 4-5 days before symptoms appear. Increasing age, pre-existing metabolic disorders, and male gender have been confirmed to be well-established risk factors for developing severe COVID-19 (51-53). These observations are, however, implicit in the ability of SARS-CoV-2 to use a specific entry, such as ACE1 and ACE2 receptor, to infect cells, tissues, and organs, so even low expression of these and other genes should not be ignored (51-53). Therefore, genetic predispositions, such as single nucleotide polymorphisms (SNPs) on particular types of genes, have also been viewed as important factors contributing to disease outcomes and must be considered in order to understand the pathophysiology of SARS-CoV-2 (53).

The presence of SNPs conducted by our team, linked loci containing haplotype variants at ACE-1 (I/D higher prevalence in COVID-19 patients), Serpina3 (G/T higher prevalence in COVID-19 patients), CRP (T/T higher prevalence in COVID-19 patients), IL6 rs1800795 (G/G-G/C higher prevalence in COVID-19 group), and IL10 (A/A higher prevalence in COVID-19 group) and IL1RN (C/T-T/T higher prevalence in COVID-19 group; C/C higher prevalence in the healthy group), IL6R (A/A lower prevalence in COVID-19 group), VDR (Fok1 TC higher prevalence in COVID-19 group; Taq1 A/G higher prevalence in COVID-19 group), IFNγ (A/A lower prevalence in COVID-19 group), and TNFα (G/G higher prevalence in COVID-19 group) (35, 38, 39).

Those mentioned SNPs have also been linked to pulmonary fibrosis and acute kidney failure, observed in severely infected COVID-19 patients. Genetic predispositions for severe COVID-19 have also been linked to genes involved in TLR3-dependent and TLR7-dependent type I interferon induction, amplification, and detection. Recent studies have found that neutralizing autoantibodies to IFNα-γ are associated with severe COVID-19. These antibodies are present in ~4% of uninfected individuals older than 70 years and have been estimated to contribute to ~20% of COVID-19-related deaths (35, 38, 39).

Of note is the correlation between PF, pCO2, pH, and some of those SNPs, which have also been proven to be associated with a critical condition in abnormal tissue biology, such as in brain tumor microenvironment. The acute hypoxemia and the consequent hypercapnia observed in COVID-19 patients requiring mechanical ventilation were observed in those individuals with ACE and VDR expressing those SNPs. The outcomes showed an increase in renal vascular resistance, which in turn contributed to renal hypoperfusion that led to acute tubular injury, AKI, myocardial damage, and brain damage (47, 49).

Adjunctive information highlighted by this study was the correlation between pO_2 and urea, the possible cause of brain damage. On the other hand, histology tests performed on lung tissues of deceased individuals with COVID-19 showed that diffuse alveolar damages were the predominant features (48-51). Lungs showed perfused vessels with large hypoxic areas in the surrounding interstices. Arterial pO_2 significantly reduces during later stages of uremia, which is one of the main traits of the latest stage of COVID-19 sepsis, characterized by a progressive metabolic alkalosis seen as a compensative mechanism followed by hypercapnia, and arterial hypoxemia, as initially reported by this article (50, 51).

We were tracking lymphocytes and neutrophils during COVID-19 infection in patients with renal failure with impaired eGFR correlated with impaired serological responses, and our results showed a significant correlation between WBC counts, creatinine, glucose, high IL-6, and CRP levels, typical features of acute myocardial inflammation (AMI) (42, 49).

Progressive lymphopenia with a relatively high number of neutrophils was also observed in association with more advanced stages of chronic kidney failure (35). On the other hand, evidence of diffuse alveolar damage in the lungs of deceased individuals with COVID-19 showed features of typical AMI and kidney failure accompanied by exudative and proliferative phases with interstitial and intra-alveolar edema, dying pneumocytes, and microvascular thrombosis (42-44).

The increased concentrations of inflammatory cytokines due to decreased renal clearance and increased IL-6 production highly contributed to respiratory failure and AKI, suggesting a lung-kidney-heart-brain crosstalk (44).

Enhanced coagulation processes are seen in post-mortem COVID-19 cases are related to the body's compensatory mechanism to limit infection by stimulating protease-activated receptors and toll-like receptors (TLRs) on macrophages, epithelial cells and endothelial cells (50, 51).

Deceased vs survivors. Significant statistical outcomes

On univariate analysis, the outcomes showed that age, oxygen saturation, RR, and systolic-diastolic blood pressure were associated with the course of the infection. Our results revealed notable ABG values and plasma kinetics patterns in both COVID-19 survivors and those deceased due to COVID-19 sepsis. As expected, clinical and hematological outcomes between the two groups showed substantial differences. Deceased patients showed increased signs of inflammatory responses and altered metabolic values with indices of clot formation above high-risk indices. Similarly, blood gas analysis confirmed a substantial impairment of gas exchange at the alveolar level.

The deceased group demonstrated a significantly lower median/mean of age (deceased-73.88 vs survivors 67.7) (p-value > 0.05), the onset time from the first symptoms was>4 days for deceased *vs* <4 days for survivors) (p-value > 0.05), The PF ratio, together with the pO_2 value, were of the best criteria to understand the profound diversity between the two groups, respectively (deceased-58.63 vs 83.92 survivors) (p-value > 0.05), and (deceased-51.15 vs 74.41 survivors) (p-value > 0.05). CRP (mg/dl) levels were also extremely different; the deceased group showed a level of 126.2 vs 72.3 of survived (p-value > 0.05). Uric acid (ml/dl) also showed differences; the deceased level was 6.8 vs 5,72 of the survived (p-value > 0.05). In addition, glucose, eGFR, IL-6, and D-dimer were also markedly different between the two groups, though not statistically significant.

It is enough to remember that just as disseminated intravascular coagulation (DIC) related to sepsis has been considered associated with an unfavorable prognosis and high mortality, the high value of the glycemic index is also always indicative of a higher mortality risk in sepsis. Although the presence of elevated glucose, altered ABG, IL-6, and elevated CRP levels along with DIC in fatal sepsis is something rare to encounter, it has been a very common scenario in severely ill patients with COVID-19 (34, 35, 38).

Furthermore, PF, hypoxia and hypocapnia, glucose variability, and high levels of inflammatory patterns are believed to be closely associated with endothelial dysfunction and cellular organ damage in vivo (lungs, liver, and kidneys). Finally, epitheliopathy is profoundly involved in the pathogenesis of sepsis, which is aggravated by aberrant activation of intravascular coagulation, leading to sepsis-related DIC, MODS, and death (4-6).

The overall impact triggered by high blood sugar levels, high D-dimer levels, hypoxia, hypocarbia, and inflammation (high CRP and IL-6 levels) were seen as the main factors that could have contributed to neurodegenerative forms at medium and long term characterized by the so-called "brain fog", paresthesias and idiopathic neuropathic pain that occur during and after the acute phase of the disease and "Long COVID" (50). These conditions were predominantly observed in older adults and individuals affected by pre-existing degenerative burdens. This causes the microglial to switch to pro-inflammatory phenotypes, resulting in a more intense inflammatory response and "inflammaging" (50-53).

Final evaluations

These findings re-emphasize that COVID-19 disease represents a complex scenario characterized by a deep inflammatory state with clear signs of irreversible, progressive decay characterized by cells, multi-level tissues, and organ damage, especially among deceased patients. A correlation is supposed to be related to the increase in cell death due to necrosis immediately preceding days due to the immense amount of purine released from cellular DNA and a reduced renal excretion due to renal, heart, and lung failure in the context of MODS. That said, COVID-19 is a disease that needs to be related to immunocompromised patients represented by a minor part of the general population. SARS-CoV-2 infection was contracted by 20–25% of subjects with inborn errors of immunity, with 75–80% having a mild or asymptomatic clinical profile (48-50).

In addition, the presence of certain bacteria as co-contributors of systemic decay and sepsis is also a matter to be discussed. The presence of these pathogens in lung fluid (BALF) together contributes to either elevated inflammatory patterns with a rise of coagulation observed in the 100% of patients admitted in 118 Units, justified the use of large spectrum antibiotics, histamines, and corticosteroids (41-44).

However, these results imply that current prophylactic dosing regimens, even if weight-based, may not be sufficient if the time of commencing treatment passed over 4 days. Given observations of enhanced platelet activation and increased platelet-neutrophil immunothrombi in COVID-19 patients, additional preventive measures were needed to reduce thrombotic risk and, therefore, sepsis associated with COVID-19. These strategies are now being tested in clinical trials, and our findings add support to the rationale and need for these studies (34, 35, 38, 51, 52).

Study limitations

In conclusion, the authors are aware that this study had some limitations. In practice, the shock index cut-offs used in this study are somewhat arbitrary, without general agreement on the ranges of these indices to predict the prognosis of sepsis. All risk stratification tools in this study were prognostic for the 7-day outcome, including ARDS, SIRS, MODS, and qSOFA criteria, into the new predictive model, the EESS. Therefore, we applied a combination of values that were verified in previous studies.

Another limitation of this study is due to its single-center study design with a small sample size, which tends to narrow and limit the generalizability of our findings to all patients with COVID-19 and sepsis. Furthermore, the retrospective design of this study may have introduced possible inherent bias in patient enrollment.

CONCLUSIONS

Ultimately, the overall results suggested that recognizing sepsis within a restricted time and critical environment such as that of the ambulance is highly variable. Most screening tools studied in clinical practice favor criteria in the hospital but not on the street. Of course, derivative screening tools have been developed, which appear to include more possibilities and should also be closely associated with the ability of the operator on site. The retrospective application of ambulance data to these evaluation and screening criteria must be supported by the theoretical and practical preparation of emergency medical personnel, which guarantees the recognition of sepsis with a higher degree of predictivity and specificity.

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Conflicts of Interest
The authors declare no conflict of interest.

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Retrospective Study

A DIFFERENT PERSPECTIVE ON SARS-COV-2 PANDEMIC: DATA, OUTCOMES AND DEMOGRAPHIC ANALYSIS OF A STUDY CONDUCTED AT GENERAL HOSPITAL NINH THUAN PROVINCE IN VIETNAM IN 2022

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ABSTRACT

SARS-CoV-2 infection was first reported in 2019 and has since spread worldwide. This is a cross-sectional study in cooperation with the School of Medicine of Aldo Moro University of Bari City, Italy. We conducted a study on how the COVID-19 epidemics evolved and how different countermeasures contained it by taking into account data showing socio-demographic and that older persons, as well as individuals with comorbidities and poor metabolic health, and people coming from economically depressed areas with lower quality of life in general, are more likely to develop severe COVID-19 infection. Examine the association between county-level socio-demographic risk factors and COVID-19 incidence and mortality, determining the possible emo-biological markers, ferritin, and lymphocytes indicative of SARS-CoV-2 infection. Methods: A descriptive cross-sectional study was conducted on 600 patients examined and treated at General Hospital Ninh Thuan from January to September 2022. Thirty-three of 600 patients were confirmed to be infected with SARS-CoV-2 (5.5%), males 4.8% and females 5.8%; the median age of infected patients is 36 years. Most infections were mild (75.8%). Our results revealed that socio-demographic arrangements' structure and spatial arrangement are important as epidemiological determinants or disease markers. Approximately 5.5% of patients infected with SARS-CoV-2 come for examination and treatment at the hospital; these findings suggested that possible infection rate in the burden of the COVID-19 pandemic, the sociodemographic risk factors, and their root causes must be addressed. In addition, lab results obtained from affected patients showed that lymphocytes and ferritin could be considered traits of mild COVID-19 infection.

KEYWORDS: *COVID-19, SARS-CoV-2, socio-demographic analysis, ferritin, lymphocytes, General Hospital Ninh Thuan province*

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INTRODUCTION

Though COVID-19 has stopped spreading around the world, we are still facing post-infection consequences, often extremely serious. More than 600 million confirmed COVID-19, including 6.564.556 deaths, were reported to the World Health Organization (WHO), Southeast Asia with more than 60 million people infected and nearly 800.000 deaths, and in Vietnam, there have been more than 11 million cases of infection with 43.000 deaths (1).

In the context of the ongoing pandemic, the spread of COVID-19 placed a significant burden on local healthcare systems and significantly contributed to high mortality rates. Timely and effective interventions were essential to reduce infection and death rates during the COVID-19 outbreak. Such interventions included rapid identification of the infections, followed by immediately applying control measures and daily monitoring procedures. An effective screening tool was based on blood tests, including white blood cells, platelets, CRP, and Ferritin (2, 3).

Scientists and labs worldwide have conducted vast research on COVID-19; the described clinical and subclinical characteristics and epidemiological features have been of great help in understanding this pathology. In Ninh Thuan Province, in general, and at the General Hospital of Ninh Thuan Province, data and outcomes were collected in quite a unique scenario. Though the reported infection rate was high, the confirmed infection numbers told a different story; numbers were relatively lower; we observed modest COVID-19 incidence despite consistent exposure at work or in the environment. Community and work contact was strongly associated with infections; however, despite the high-risk exposure, the estimated rate of COVID-19 patients coming for examination and treatment was no more than 14%, as confirmed by similar studies around the world (4).

Therefore, with this paper, we have tried to answer the following question: what was the actual rate of SARS-CoV-2 infection in patients who come for examination and treatment at General Hospital Ninh Thuan Province? To answer the above question, we conducted research on the topic with the following two objectives:

1. determine the prevalence of patients positive for SARS-CoV-2 at the General Hospital of Ninh Thuan Province in 2022, with a general focus on the importance of socio-demographic patterns;
2. surveying concentration of some subclinical tests in patients positive for SARS-CoV-2 with a general focus on lymphocytes and ferritin patterns in low-medium severe infected patients.

MATERIALS AND METHODS

Subjects of study

All patients were hospitalized at General Hospital Ninh Thuan Province from January 1 to September 2022.

Selection criteria:

All patients hospitalized at General Hospital Ninh Thuan Province from January 1 to September 2022 suspected of having COVID-19 are indicated for SARS-CoV-2 test (rapid or RT-PCR).

Exclusion criteria:

The patient was negative (rapid test or RT-PCR) at the following tests during patient follow-up.

Study Design:

Cross-sectional study Sample size. Minimum sample size: $Z^2(1\text{-}\alpha/2) = 1{,}96$; p = 0,15 [5]; d = 0,03; the sample size we calculated is 544, but the actual number of patients studied is 600.

$$n = \frac{Z^2_{(1-\alpha/2)}\, p(1-p)}{d^2}$$

Sampling method

Convenience sample selection, consecutive.

Statistical analysis

SPSS 22.0 statistical software was used for all calculations. Qualitative variables are expressed as percentages (%). Use medians and percentile ranges (25 and 75) to describe quantitative variables that are not normally distributed.

Chi-square test, Fischer-exact test for categorical variables, student's t-test, or Mann Whitney U test for continuous variables as appropriate. $P < 0.05$ were considered as statistically significant.

RESULTS

Association of population socio-demographic condition and health care measures with COVID-19 Incidence

Most of the population's socio-demographic data, such as age, gender, or healthcare availability measures adopted by the Government bodies, were significantly associated with lower COVID-19 incidence (Tables I–V). Age rate was significantly associated with COVID-19 incidence (IRR, 1.02; 95% CI, 1.01-1.02; *P* >0.05). As expected, life expectancy was significantly associated with mortality, such that an additional year was associated with lower mortality rates (IRR, 0.95; 95% CI, 0.93-0.97; *P* >0.05).

TABLE I. *Demographic characteristics of the study (N=600).*

Characteristics	Frequency	Percentage
Gender		
Male	188	31.3
Female	412	68.7
Age group		
0 - 9	40	6.7
10 - 19	45	7.5
20 - 29	161	26.8
30 - 39	143	23.8
40 - 49	53	8.8
50 - 59	56	9.3
60 - 69	58	9.7
70 - 79	29	4.8
≥ 80	15	2.5
Median age (25^{th} - 75^{th}): 32 (24 - 51) years old		

TABLE II. *Prevalence of SARS-CoV-2 positivity of gender***.**

Gender	Number tests	Positive	(%)	OR (95% CI)	*P*
Male	188	9	*4.8*	0.8 (0.4 - 1.8)	0.6
Female	412	24	*5.8*		
Total	**600**	**33**	***5.5***		

TABLE III. *Prevalence of SARS-CoV-2 positivity age group.*

Age group	Number tests	Positive	Percentage
0 - 9	40	2	5.0
10 - 19	45	1	2.2
20 - 29	161	6	3.7
30 - 39	143	12	8.4
40 - 49	53	4	7.5
50 - 59	56	1	1.8
60 - 69	58	4	6.9
70 - 79	29	2	6.9
≥ 80	15	1	6.7
Total	**600**	**33**	**5.5**

TABLE IV. *Disease severity and treatment outcome (N=33).*

Characteristics	Frequency	Percentage
Disease severity		
Asymptomatic	0	0.0
Mild	25	75.8
Moderate	7	21.2
Severe	1	3.0
Critical	0	0.0
Treatment outcome		
Recover	33	100
Death	0	0.0

TABLE V. *Concentrations of some laboratory tests.*

Tests	Total (n=33)		Mild (n=25)		Moderate and Severe (n=8)		*P*
White Blood Cell (/mm^3)							
< 4000	0	0.0	0	0.0	0	0.0	0.05
4000 - 10.000	31	93.9	25	100	6	75.0	
>10.000	2	6.1	0	0.0	2	25.0	
Median	4.600		4.500		7.500		<0.01
25th - 75th	4.400 - 5.000		4.300 - 4.600		6.000 - 13.500		
Lympho (/mm^3)							
< 1.500	18	54.5	11	44.0	7	87.5	0.04
Median	1.100		1.600		700		<0.01
25th - 75th	800 - 2.000		1.100 - 2.000		625 - 800		
Platelet Count (/mm^3)							
< 150.000	20	60.6	14	56.0	6	75.0	0.3
Median	180.000		180.000		205.000		>0.05
25th - 75th	127.500 - 200.000		120.000 - 200.000		147.500 - 243.750		
Hemoglobin (g/dl)							
Median	12.2		12.2		12.6		>0.05
25th - 75th	11.0 - 13.1		11.1 - 13.2		11 - 13		
CRP (mg%)							
CRP >10	3	9.1	1	4.0	2	25.0	0.1
CRP ≤ 10	30	90.9	24	96.0	6	75.0	
Ferritin (ng/mL)							
Median	200		170		300		<0.01
25th - 75th	150 - 275		145 - 256		273 - 348		

Laboratory tests

In this study, the median white blood cell count in patients with COVID-19 was 4.600, lymphocytes 1.100, platelets 180.000, hemoglobin 12.2 g/dl, CRP >10 mg% only 9.1%, ferritin 200 ng/mL. There was a statistically significant difference in the median number of WBC, lymphocytes, and ferritin between the 2 groups, mild and moderate with severe ($P < 0.05$). According to Chen et al. (2), the whole blood count on admission of 3 (30%) moderate cases showed mild leucopenia. In contrast, white blood cell counts were normal or slightly increased above the upper limit of normal in all the severe cases. In contrast, lymphocyte counts were significantly lower in severe cases (0.7 × 109/L) than in moderate cases (1.1 × 109/L). Lymphopenia (lymphocyte count <0.8 × 109/L) was developed in 8 (72.7%) severe cases and only 1 (10.0%) moderate case ($P = 0.008$). Overall, severe cases had increased WBC counts ($P = 0.003$) but lower lymphocyte counts ($P = 0.049$) (Fig. 1).

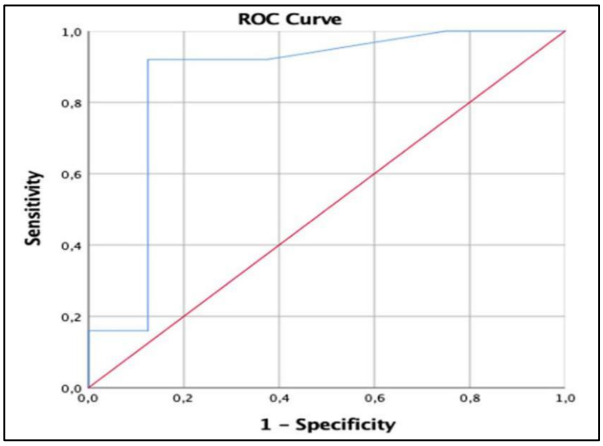

Fig. 1. *Lymphocyte value determines disease severity.*

Studies showed that the median platelet count in patients with COVID-19 was 163 x 109 /L, and there was no significant difference (*P* >0.05) in the ICU and non-ICU groups. The median hemoglobin in patients with COVID-19 was 13.4 g/dl, and no difference was found between severe and non-severe cases. However, CRP ≥ 10 mg/l accounts for 60.7% in a study by Guan et al., our CRP >10 mg% only accounts for 9.1% (Table IV) (5-7). The reason there is a difference here is that the author's research was conducted in the early stages of the COVID-19 epidemic, and no patient was vaccinated against COVID-19; at the same time, in this study, the author, there were many patients with severe disease, the mortality rate was 1.4% (5). In our study, only 1 patient (3.0%) had severe severity and a mortality rate of 0.0% (Table III). Chen et al. (2), ferritin concentration in severe cases was significantly higher than in moderate cases (*P* < 0.05) (Fig. 2).

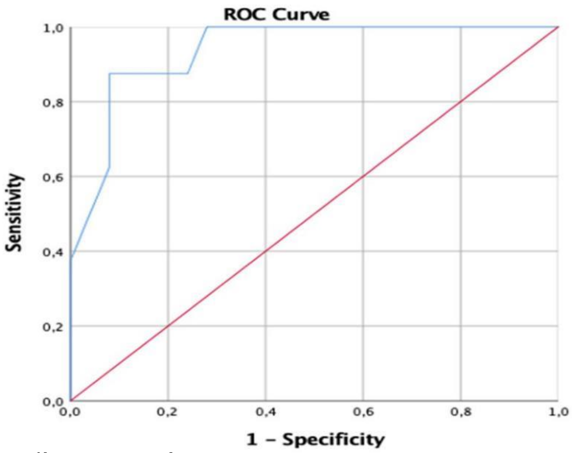

Fig. 2. *Ferritin value determines disease severity.*

Prevalence of positive for SARS-CoV-2 in patients examined and treated at the Hospital

Females accounted for 68.7%, mainly in the age group of 20-29 years (26.8%) and 30-39 years old (23.8%). The prevalence of patients positive for SARS-CoV-2 was 5.5% (95% CI: 0.3 - 7.6) in the overall sample. Prevalence positive in females was 5.8%, and in males was 4.8% (*P* >0.05).

The positive prevalence was highest in ages 30-39 (8.4%) and 40-49 (7.5%). The median age of the positive group was 36 years, and the negative group was 32 years (IRR, 1.02; 95% CI, 1.01-1.02; *P* >0.05).

Most COVID-19 patients had mild disease (75.8%), 1 patient was severe (3.0%), and no patient was in critical condition. The recovery rate at Ninh Thuan Provincial General Hospital is 100%.

The median white blood cell count in patients with COVID-19 was 4.600, lymphocytes 1.100, platelets 180.000, hemoglobin 12.2 g/dl, CRP >10 mg% only 9.1%, ferritin 200 ng/mL. There was a statistically significant difference in the median number of WBC, lymphocytes, and ferritin between the 2 groups, mild and moderate with severe (*P* < 0.05) (Table V).

DISCUSSION

Prevalence of positive for SARS-CoV-2, a socio-demographic point of view

This study aimed to determine the relationship between socio-demographic factors, adherence to social recommendations, and the effective rate of positive cases of COVID-19 during the pandemic in Ninh Thuan General Hospital of Ninh Thuan Province, Vietnam. The results showed that positives were closely adherent to specific social-demographic traits of Vietnam and the possible distancing recommendations ruled by the Central Government and Provincial Health bodies. Despite the high adherence rate to social distancing recommendations in the whole population, low infection rates were observed concomitantly to precise socio-demographic patterns that directly impacted the response to COVID-19 in terms of the numbers of infections, such as age. According to the multiple linear regression model, age was the most important determinant of adherence to behavioral recommendations, followed by occupation, gender, awareness, and history of metabolic illness (8).

Therefore, the need for this analysis was also to determine the influences of socio-demographic variables in contributing to the increased number of COVID-19 cases in different ″hotspot″ areas of the world. With such information, the authorities may easily understand, develop, and implement the needed measures to minimize and handle the increased number of cases in these areas. This would help in settling and planning the resources more feasibly, such as the creation of quarantine sites, distribution of informative pamphlets and countermeasures to be adopted by the population, and the building of COVID-19 health-care spots to help contain and the spread of the virus (8).

The socio-demographic planning plays a crucial role in revealing the pattern of COVID-19-positive cases and deaths globally (9, 10). The aging factor plays a crucial role in controlling COVID-19 deaths and spreading. For instance, the high number of COVID-19 deaths and infections in Italy has to be associated with demographic values. Italy's median age of the population is 46.5 years, with almost a quarter being over 65. Interestingly, according to the World Health Organization, age was a key factor, at least during Europe's first two waves of COVID-19 infection (7, 8). More than 95% of people deceased were over 60; comorbidities are usually something related to old age; therefore, long-time illness and existing disease history are also found to be associated with COVID-19 deaths. A USA study found that occupations also have had a significance in explaining a few aspects related to the COVID-19 pandemic, as people with high-level social status and more frequent social interaction were more vulnerable to being infected with the virus (8).

Many studies have found people with metabolic conditions such as diabetes to be at an increased risk of COVID-19 compared to individuals without diabetes (11). In general, at least during the first period of the pandemic, males and older adults were included among the risk factors associated with COVID-19; however, successively, other results showed that the positive prevalence of SARS-CoV-2 in females (5.8%) and in males (4.8%) were almost equally distributed (P>0.05) (12). Similarly, we found no association between age and SARS-CoV-2 in Vietnam but not Italy. Our group in Italy showed compatible results with Setiadi et al. during the same period; the outcomes showed that the prevalence of COVID-19 was higher in the group >60 years old (29.6%), followed by the group 41- 60 years old (24.2%) and lowest observed in children under five years (11.0%) (13, 14). At the beginning of the pandemic, the elderly were the most vulnerable to infection as the risk of infectivity increases with age (15-17).

As the pandemic progressed, many studies found evidence that younger adults likely contribute to community transmission of COVID-19 (18). Different studies confirmed a switch in age trend primarily seen in youngsters and adults <65 that could be seen in the late stage of the COVID-19 pandemic (19). The explanation may be that these groups dominate the population size and case numbers in Western countries, China, and South America. In particular, they are more engaged in social activities than other age groups. Some authors found that during May-August 2020, the median age of COVID-19 cases in the US declined from 46 years in May to 37 years in July and 38 years in August. People aged 20-29 years accounted for the most significant proportion of total cases (>20%) during June-August (19, 20). In our research, the median age of the positive group was 36 years, and the negative group was 32 years (P >0.05).

Despite its small land surface area, Vietnam is one of the world's most densely populated areas. However, the Ninh Thuan region is one of the lowest densely populated in Vietnam, with just 178.000 people/sq km in 2021 and a total population of 679,467; the region′s female and male population is practically equivalent. More than half of the population are under age 38 and from the working class, fishing, and agriculture.

Even though international migration is low, migrants to big cities such as Ho Chi Minh City, Danang, and Hue outnumber those outside the area. We agreed on the fact that despite those studies' significant advancements, most of them included participants irrespectively of their potential symptomatology, the specific prevalence of different COVID-19 variants, the associated symptoms (e.g., cough, fatigue, aches, ageusia, anosmia or fever), and the country's population average age. We must point out that the average age between the different areas plays a crucial role in assessing and understanding the data. Vietnam, for instance, has a young population compared to Germany and Italy; more than half of the people living in Vietnam are under 35 years old, which is very close to the USA population, which seems to be under

38 years old. Due to this, the sociodemographic and clinical characteristics of individuals diagnosed with COVID-19 and the general outcomes may explain current conflicting findings (Fig. 3-5).

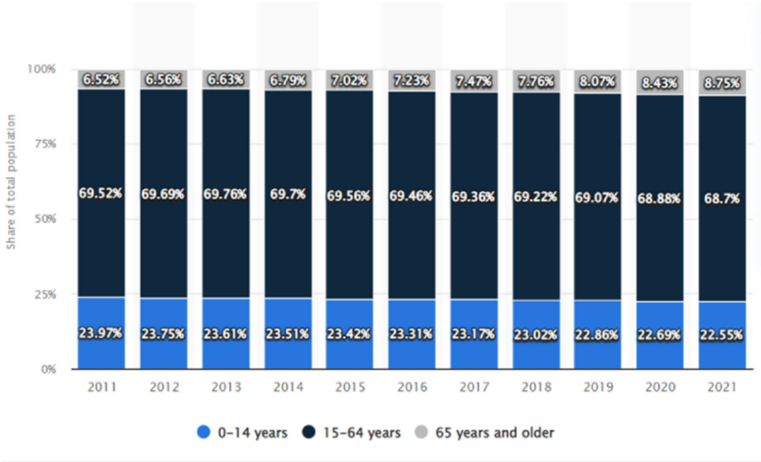

Fig. 3. *Vietnam population average age.*

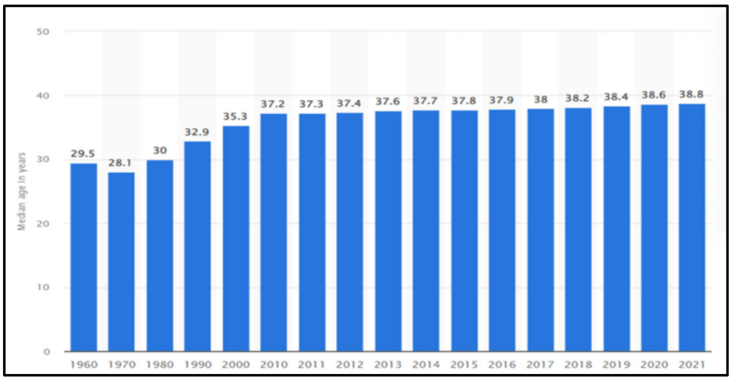

Fig. 4. *USA population average age.*

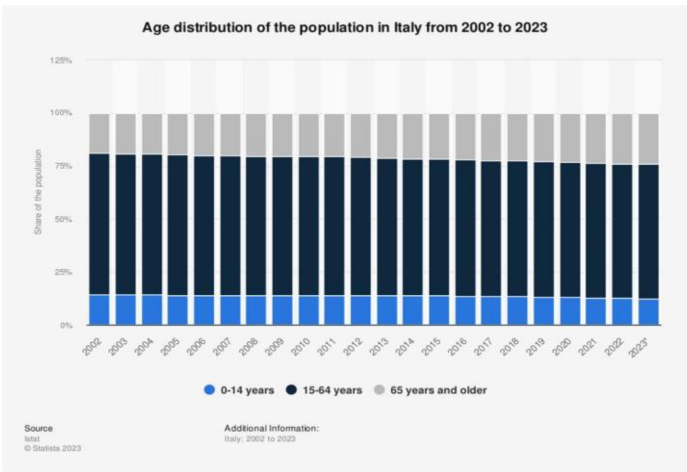

Fig 5. *Italian population average age.*

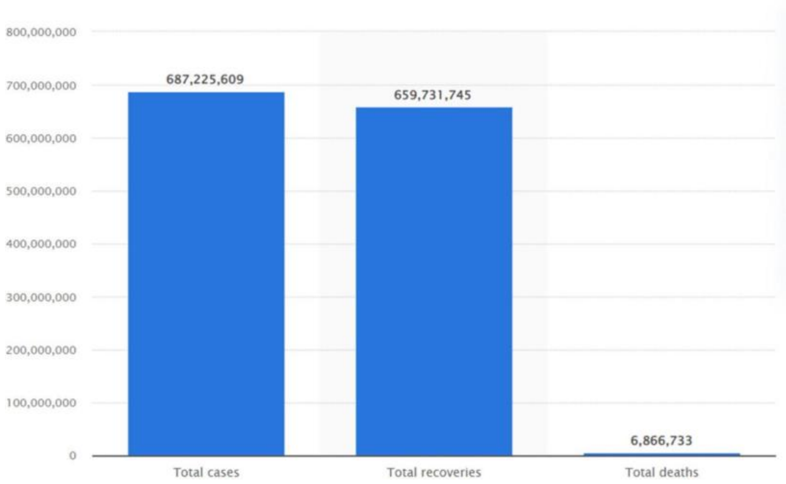

Fig. 6. *Number of coronavirus (COVID-19) cases, recoveries, and deaths worldwide.*

In this study, the prevalence of patients positive for SARS-CoV-2 was 5.5% (95% CI: 0.3 - 7.6) in the overall sample (Table II). Situations worldwide told sometimes different stories. In a study by Setiadi et al., of the 64,364 swab specimens tested in Indonesia, 15.7% (n = 10,130) were positive for SARS-CoV-2 (14). Balzanelli et al. and Jacob et al., respectively, in Italy and Germany, the prevalence of symptomatic patients monitored in their hospital facilities were approximately the same, 13.8% and 17% (4, 13-15); meanwhile, the results of Mani et al. (21). In Seattle-USA, the positive rate of SARS-CoV-2 was 5.3%, similar to ours in Vietnam (5.5%). In this study, the general distribution of affected patients showed that females accounted for 68.7%. The male was 31.3%, mainly in the age group of 20-29 years old (26.8%) and 30-39 years old (23.8%); the median age was 32 years, prevalence and some related factors in symptomatic patients monitored at general hospitals in Germany, female accounted for 54.7%, and the mean age was 44.6 years (22).

On the other hand, our group in Italy showed quite a different scenario; the patients reporting the COVID-19 infection symptoms were divided into these age groups, indicating a net majority of males compared to females (74 vs 26%), 0-9 years (1.70%), 10-19 years (3.80 %), 20-34 years (13.19 %), 35-59 years (36.66 %), 60-69 years (14.29 %), 70-79 years (15.08 %), 80-89 years (11.99%), ≥ 90 years (3.30%).

This Vietnamese study showed that approximately one in twenty symptomatic patients tested for COVID-19 was diagnosed with COVID-19. It is difficult to compare this finding with previous studies′ results, as most of these studies included symptomatic and asymptomatic participants, were conducted in other settings and countries, and were conducted independently from different COVID-19 variants. If we compare these results with those from other studies, we may conclude that numbers refer to a condition often overestimated, as many were false positives and false negatives. For instance, a U.S. study of 3,477 symptomatic healthcare workers reported that 5.3% of the sample had COVID-19. Another study using samples from 2,203 individuals from Germany collected in May–June 2020 found that none of the throat swabs taken was positive for SARS-CoV-2 (4, 22). Worldwide, the numbers seem to confirm this trend, with 680 million people affected vs 670 million recovered with a rate of mortality of almost 7 million (8,56%); in Asia, only 217 million were infected with a mortality of 1.5 million c.ca (4.56%) (Fig. 6) (1).

With the correct proportions, the current study showed that the prevalence of COVID-19-infected individuals appears to be relatively low, 33 out of 600. These findings highlight two main points: (i) the age, the younger, the lesser the possibility of catching the infection; (ii) the important role played by general awareness campaigns either in prevention adopted measures or (iii) in earlier diagnosing procedures conducted by the health authorities in the Ninh Thuanh province in particular and in Vietnam in general, stressing out the crucial role of implementing preventive measures in controlling the transmission of the virus in the communities and hospitals.

In our study, the majority of COVID-19 patients had mild disease (75.8%), 1 patient was severe (3.0%), and no patient was in critical condition (Table III). Because most of the infections were mild, the rate of patients recovering at Ninh Thuan General Hospital is very high (100%). This result is consistent with the current situation, though almost all patients have received the 2nd and 3rd doses of the COVID-19 vaccine. Still, most of the critically ill patients are elderly, accompanied by underlying disease. The median age of patients in our study was young (36 years), so a high cure rate is appropriate.

The second result from the present study was the characterization of possible adjunctive markers that may refer to COVID-19 infection; therefore, those blood markers could be significantly associated with the differences involved with the disease tendency. Significant physiognomic differences exist in the human immune system response against SARS-CoV-2 at various stages of the infection (e.g., virus entry, virus sensing, innate immune response, etc.) and towards the different types of its variants. These differences may result in a higher risk of COVID-19 or more persistent detection of viral RNA in different individuals independent of gender, age, or factors.

From a clinical perspective, previous research has also shown that compliance with preventive public measures is lower in men than in women, which may at least partially explain the relationship between gender and COVID-19 diagnosis observed in this study. Finally, several age-related changes, such as immunosenescence, inflammation, and a dysregulated renin-angiotensin system, may increase COVID-19 susceptibility in older adults compared to their younger counterparts. Furthermore, in line with previous research, there was a positive association between the male gender, older age, and the severity degree of the disease. However, the strength of the association between the different parameters, such as gender, the possible presence of specific genetic make-up, such as the single nucleotide polymorphisms (SNPs), and the diagnosis of COVID-19 was considered a strong determinant variable (i.e., OR 1.04) (8, 22, 23).

It is generally accepted that individuals are differentially affected by COVID-19. Although pre-existing disorders have been studied extensively, little is known about how much the genetic background may influence the predisposition to the disease. The rapid progression of the infection accompanied by a sudden clinical decay seemed a prerogative of certain subjects rather than others (11, 24-28). The excessive uncontrolled inflammatory responses mainly determined by the overexpression of typical inflammatory factors and markers such as IL-6, IFNγ, and TNF-α were common clinical traits of this infection. There was little or no homogeneity among patients. Among the unpredictability of the events, this trait drove attention to the possible role of single nucleotide polymorphisms (SNPs) of those genes involved in the immune regulatory mechanism. The disease severity was soon observed in connection to several genes carrying specific SNPs (28-30).

For instance, while the IL6 174 G/G genotype indicates an overexpression of IL6 expression, the G/C is mainly related to moderate IL6 expression. In addition, it is known that IL6 may become completely unmanageable in the presence of low circulatory level of IL10 as a consequence of IL6 gene down-expression, indicated by SNPs with the genotype A/A, which characterize the SARS-CoV-2 “cytokine storm”. Thus, the SNPs′ analysis may also play a vital role in the diverse degree of infection and the differences in individual responses to Sars-CoV-2 (23-25).

Hyperferritinaemia has been considered an indicator of inflammatory processes in different disorders. Hyperferritinaemia may be due to leakage from damaged intracellular stores, and once released from tissue stores, ferritin loses the inner iron content, allowing the rise of free iron, which also favors the growth of many viruses (26-28).

Iron metabolism and immune response to SARS-CoV-2 have not been fully described, although several lines of evidence demonstrated their involvement in COVID-19 pathogenesis. Our research showed that the median ferritin in patients with COVID-19 was 200 ng/mL; there was a statistically significant difference in the median ferritin concentration between the two groups (P <0.05), AUC=0.9, P<0.001. Many studies confirmed the high presence of ferritin concentration with moderate and severe disease in patients with COVID-19; our results showed a cutoff of 266 ng/ml, a sensitivity of 88%, and a specificity of 92% (29).

Lymphopenia and specific T cell lineage affection are characteristic features of COVID-19 and have been correlated with poorer prognosis (26-28).

In previous coronavirus outbreaks, such as SARS, the peak of viral load occurred 7 days after symptoms development, followed by elevation in IL-6 and IL-8, low lymphocyte count, high neutrophils count, and subsequent neutrophils pulmonary infiltrates. This description suggests that clinical symptoms might be mediated by the immune system deregulation rather than direct viral damage (17, 30). The distribution of different subtypes of T cells in the peripheral blood of symptomatic critical and non-critical COVID-19 patients has been described (30-32).

In our experience, the majority of patients affected by COVID-19 shared a quite common scenario characterized by important high levels of IL-6 and CRP presented with increased serum fibrinogen and troponin with low levels of eGFR and hemoglobin and hematocrit, with slightly high levels of RDW (suggestive of iron anemia) (30-32).

Different studies demonstrated the capacity of the Sars-CoV-2 virus to disrupt the coordination between the two branches of the immune system, innate and adaptive immune responses. The affected patients′ immune profiles showed low levels of B-lymphocytes, low levels of T-regs CD4+CD25+, high levels of T CD8+CD38+DR, high levels of T-suppressor CD8+CD57+, high levels of T-NK CD3+CD56+ (higher than NP, NN, and NA). High levels of neutrophils, low levels of lymphocytes, low levels of T-mature CD3, low levels of CD4, low ratio < 1 CD4/CD8, low levels of naïve CD4 (30-32).

CONCLUSIONS

In this cross-sectional study, a wide range of sociodemographic risk factors, including age and environmental factors, were significantly associated with COVID-19 incidence and mortality. To address inequities in the burden of the COVID-19 pandemic, these social variabilities and their root causes must be addressed. Approximately 5.5% of patients infected with SARS-CoV-2 come for examination and treatment at the hospital. Lymphocytes and ferritin were valuable in determining moderate and severe disease in patients with SARS-CoV-2 infection.

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Review

AMCOP® ELASTODONTIC DEVICES IN ORTHODONTICS: A LITERATURE REVIEW

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ABSTRACT

This study aims to provide a comprehensive overview of the current literature on AMCOP® elastodontic devices, focusing on their applications, advantages, and limitations in contemporary orthodontic practice. A literature review was conducted using databases such as PubMed and Scopus, covering studies published from 2010 to 2023. Search terms included "elastodontics," "AMCOP® devices," and "orthodontic elastomers". Inclusion criteria were studies focusing on the clinical application of AMCOP® devices, articles discussing the biomechanical principles of elastodontics, and reviews and clinical trials evaluating the effectiveness of elastodontic treatment. The review identified five relevant studies. These studies focused on therapies with AMCOP® devices for treating various malocclusions, including Class II and III discrepancies, open bites, and crossbites. Notable findings included overjet, overbite, crowding, and palatal symmetry improvements. Additionally, AMCOP® devices contributed to correcting hyperdivergent Class II malocclusion and enhanced upper airway space. AMCOP® elastodontic devices represent a significant advancement in orthodontics, offering a less invasive, patient-friendly alternative to traditional devices. Their use of elastic materials provides continuous, gentle forces that align with physiological tooth movement, enhancing patient comfort and compliance. However, further research, including long-term clinical trials, is essential to establish their efficacy and explore new clinical applications fully.

KEYWORDS: *elastodontic appliances, AMCOP® bio-activators, interceptive treatment, orthodontic elastomers, early treatment, orthodontic materials, functional therapy, thermo-activable materials, orthodontic appliances*

INTRODUCTION

Elastodontics is a branch of orthodontics that focuses on the use of elastic materials and devices to correct dental and skeletal discrepancies (1, 2). Unlike traditional orthodontic treatments that rely on rigid materials such as metal

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brackets and wires, elastodontics employs elastomeric appliances to exert gentle, light forces on teeth and periodontal tissues and increase phosphatase alkaline (3-5). This approach capitalizes on the biomechanical properties of elastomers, which are materials known for their elasticity and ability to return to their original shape after deformation (6). These properties make elastodontic appliances (EAs) suitable for applying controlled, sustained forces that facilitate desirable orthodontic movements (7, 8).

The concept of elastodontics is grounded in the understanding that low-force application can be more physiologically compatible with the tissues involved in orthodontic treatment (1, 9-11). It minimizes the risk of root resorption and enhances patient comfort, potentially leading to improved compliance (2). The gentle forces generated by elastomeric devices align well with the natural biological processes of bone remodeling and tooth movement, promoting efficient and effective orthodontic correction (1, 12, 13).

The Multifunctional Cranio-Occluso-Postural Harmonizers (AMCOP®) have emerged as a significant innovation in this field (1). These devices are designed to provide effective, patient-friendly solutions for various orthodontic issues, ranging from simple dental alignments to more complex skeletal corrections (14, 15). AMCOP® devices are characterized by their use of elastomeric materials, which are both flexible and durable (16). This combination of flexibility and durability allows for a wide range of therapeutic applications, making AMCOP® devices versatile tools in the orthodontist's arsenal (17).

AMCOP® bio-activators are intended to balance the dental-cranial-facial structures by addressing multiple dysfunctional disorders using a multifunctional approach. AMCOP® devices work on the link between the skull and jawbones and the interaction of occlusion and posture, focusing on the first cervical vertebrae (1, 18, 19).

AMCOP® devices are noted for their softness, comfort, and flexibility to various arch types. They are made of an elastic and thermo-activable material from a polymer-elastomer combination. During treatment, the devices can be extended by immersing them in hot water at 70°C for 30 seconds, and they can also be customized with heat-appropriate tools (20-22). The transverse distance between the outermost points of the vestibular cusps of the first two upper molars is measured to determine the appropriate size of the activator, either on a plaster model or a wax bite, with the latter being especially useful for young children who dislike taking impressions (23-25).

Each AMCOP® device has two high flanges on the vestibular and lingual sides, which prevent any muscular interference with the teeth. A central section free of indentures allows tooth mobility, while a lingual ramp and button direct the tongue onto the palate (1, 26, 27). The devices come in various colors, corresponding to different arch types and skeletal classes (Fig. 1).

Fig. 1. *AMCOP® devices (source: www.amcop.it/indicazioni, accessed on 20 May 2023).*

AMCOP® elastodontic devices are typically customized to fit the specific needs of each patient. They can be used to treat a variety of malocclusions, including Class II and Class III discrepancies, open bites, and crossbites (28, 29). The customization aspect is crucial, as it ensures that the devices can effectively address the unique anatomical and orthodontic requirements of individual patients (30-33).

These devices often feature a combination of soft and hard elastomers strategically arranged for differential force application. This design allows for targeted tooth movements, improving the precision and efficacy of the treatment. Additionally, AMCOP® devices can be designed to encourage natural jaw movements and muscle function, contributing to more stable long-term results (34, 35).

The primary aim of this review is to provide a comprehensive overview of the current literature on AMCOP® elastodontic devices. By synthesizing the findings from various studies and clinical reports, this review aims to highlight the applications, advantages, and limitations of AMCOP® devices in contemporary orthodontic practice.

MATERIALS AND METHODS

A literature review was conducted to gather relevant information on AMCOP® elastodontic devices. The search strategy included terms such as "elastodontics," "AMCOP® devices," and "orthodontic elastomers". The databases searched were PubMed and Scopus, covering studies published from 2010 to 2023.

The inclusion criteria were:

- studies focusing on the clinical application of AMCOP® elastodontic devices;
- articles discussing the biomechanical principles of elastodontics;
- reviews and clinical trials evaluating the effectiveness of elastodontic treatment;
- English language;

Exclusion criteria included:

- studies not related to elastodontics or AMCOP® devices;
- articles published before 2010.

RESULTS

The research strategy identified five relevant articles that were included in the analysis. These articles provide valuable insights into the clinical applications and effectiveness of AMCOP® elastodontic devices in various orthodontic treatments (Table I).

Table I. *Selected items.*

Authors	Year of publication	Materials and Methods	Outcomes
Lo Giudice et al. (36)	2022	Class II AMCOP® bio-activator for one hour each day for a year and at night.	AMCOP® treatment resulted in an improvement in anterior dental crowding as well as a decrease in overjet and overbite.
Lo Giudice et al.(37)	2023	AMCOP® Integral/Basic activator for two hours each day and at night for a year.	AMCOP® treatment resulted in a reduction of palate asymmetry and an increase in palate size in children with a crossbite.
Fichera et al. (38)	2021	AMCOP® second class, one hour each day for a year, and at night. Patients were required to bite the device during the day while maintaining lip contact.	Crowding, overjet, and overbite were reduced as a result of AMCOP®. The number of Class I relationships increased.
Inchingolo et al. (26)	2022	AMCOP® in 16–18 month-old hyperdivergent children. For 6 to 8 months, AMCOP® was taken only at night and for an hour each day.	The overbite and hyperdivergence were reduced as a result of the device. Additionally, the breadth of the upper ways was improved.
Patano et al. (39)	2023	AMCOP® treatment for 3 years.	AMCOP® resulted in an enhancement in the upper way dimensions.

DISCUSSION

Recent studies have highlighted the effectiveness of elastodontic devices in various orthodontic treatments, demonstrating their potential to address malocclusions and promote harmonious craniofacial development.

The prospective clinical study conducted by Lo Giudice et al. (36) evaluated the effectiveness of elastodontic devices in treating subjects with Class II sagittal discrepancy in mixed dentition. A treatment group of 19 subjects received elastodontic devices for one year, while a control group of 17 subjects remained untreated. The results demonstrated a significant reduction in overjet and improvement in overbite in the treated group, whereas the control group exhibited a slight, non-significant increase in these parameters. Additionally, the number of subjects with anterior crowding decreased in the treated group, in contrast to a slight increase observed in the control group. 3D analysis of intraoral scans revealed pre-treatment palatal asymmetry that partially improved in the treated group compared to controls. In conclusion, elastodontic devices effectively mitigate early signs of malocclusion in Class II subjects and promote harmonious palatal development (7, 36, 40).

Building on these findings, Lo Giudice et al. conducted another study (37) to evaluate the palatal dimensional and morphological changes following the treatment of functional posterior crossbite (FPXB) using EAs. A treatment group (TG) of 25 subjects received EA treatment for one year, while a control group (CG) of 14 subjects remained untreated. Results demonstrated a significant increase in inter-canine and inter-molar widths in the TG and a reduction in the asymmetry between the crossbite and non-crossbite sides. 3D deviation analysis showed a substantial improvement in palatal symmetry in the TG, with the matching percentage between original and mirrored models increasing from 81.12% to 92.32%. Conversely, the CG showed no significant changes. The study concluded that EAs effectively correct FPXB and promote the harmonious development of the palate in children (41-43).

Furthermore, the retrospective study by Fichera et al. (38) assessed the skeletal and dentoalveolar changes following one year of treatment with elastodontic appliances (EAs) in children showing early signs of malocclusion. The study included 20 treated subjects and 20 control subjects. Digital impressions and lateral cephalograms were taken before treatment (T0) and after one year (T1). In the treated group, significant improvements were noted in overjet, overbite, crowding, and sagittal molar relationships. Cephalometric analysis showed no significant changes in SNA (sella-nasion-A point) angle values but significant increases in SNB (sella-nasion-B point) angle ANB (A point-nasion-B point) angle values. Conversely, the control group showed a slight rise in overjet and overbite without significantly improving other parameters. The study concluded that EAs effectively improve malocclusion indicators and promote harmonious dentoalveolar development, offering a viable interceptive treatment option, especially for patients with limited financial resources (44-46).

In a different but related investigation, the retrospective study by Inchingolo et al. (26) evaluated the effectiveness of elastodontic therapy using AMCOP® devices in treating hyperdivergent Class II malocclusion in children and its impact on upper airway patency. The study included 21 patients with hyperdivergent growth patterns and Class II malocclusion. Cephalometric analysis before and after treatment showed correction of the Class II malocclusion, a reduction in divergence, and improved upper airway space. Significant skeletal changes included mandibular advancement and reduction in the ANB angle. The AMCOP® devices effectively corrected hyperdivergent Class II malocclusion and enhanced upper airway space, indicating their potential in interceptive orthodontics for growing patients (1 , 46, 47).

Finally, Patano et al. (39) investigated the impact of EAs on the pharyngeal airway space and hyoid bone position in patients with skeletal Class II malocclusion. The findings demonstrated that treatment with the AMCOP® elastodontic device resulted in significant clinical improvements. Specifically, there was a notable enhancement in the superior upper airway space and the vertical position of the hyoid bone (39, 48-50). These changes suggest that elastodontic therapy can effectively increase airway dimensions, potentially alleviating breathing issues associated with Class II malocclusion. The study underscores the utility of elastodontic devices in promoting favorable orthopedic and functional modifications, contributing to better respiratory function and overall craniofacial harmony in growing patients (39, 51, 52).

These studies underscore the efficacy of elastodontic devices in addressing various orthodontic issues, promoting favorable orthopedic and functional modifications, and contributing to better respiratory and craniofacial outcomes in children.

Despite the promising results obtained, these studies present several limitations. Firstly, much of the research is retrospective, which can introduce selection bias and limit the ability to establish direct causality. Additionally, the sample size in some studies is relatively small, reducing the generalizability of the findings. The reliance on patient compliance with therapeutic guidelines represents another limitation, as non-adherence can negatively influence outcomes. Finally, most studies utilize two-dimensional cephalometric analyses, which may not fully capture the complex three-dimensional dynamics of structural changes. Future research with larger sample sizes, prospective designs, and three-dimensional analyses could provide a more comprehensive and robust understanding of the long-term efficacy of elastodontic devices.

CONCLUSIONS

AMCOP® elastodontic devices represent a valuable addition to orthodontics, offering a comfortable and effective alternative to traditional braces. Their innovative use of elastic materials to provide gentle forces aligns well with the principles of physiological tooth movement, potentially leading to better patient experiences and outcomes. The flexibility and resilience of the elastomers in these devices contribute to effective treatment while minimizing discomfort and adverse effects commonly associated with conventional orthodontic methods.

The design and functionality of AMCOP® devices promote natural jaw movements and muscle function, enhancing treatment stability and long-term results. Furthermore, their aesthetic appeal, being less noticeable than traditional metal braces, makes them an attractive option for patients concerned about the visual impact of orthodontic appliances. This is particularly advantageous for adults seeking discreet orthodontic treatment.

Further research is essential to fully establish the benefits and potential drawbacks of AMCOP® elastodontic devices in various orthodontic scenarios. Long-term clinical trials are particularly needed to provide comprehensive data on the durability, effectiveness, and stability of results achieved with these devices over extended periods. Such studies would also help refine treatment protocols, identify potential complications, and optimize patient selection criteria.

Additionally, future research should explore integrating AMCOP® devices with other orthodontic and dental technologies. For example, combining EAs with digital orthodontic planning tools or advanced imaging techniques could enhance treatment precision and predictability. Investigating the potential of AMCOP® devices in interdisciplinary treatment approaches, such as in conjunction with maxillofacial surgery or periodontal therapy, could further expand their clinical applications and benefits.

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Conflicts of Interest
The authors declare no conflicts of interest.

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Review

CROWDING TREATMENT WITH ALIGNERS: STRATEGIES AND PREDICTABILITY-A SYSTEMATIC REVIEW

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ABSTRACT

Transparent aligners offer a viable alternative to fixed appliances for correcting malocclusions, providing advantages in tooth movement, anchorage control, and specific applications such as crossbite correction and dental arch expansion. Our research aims to evaluate the resolution of crowding in aligner therapy, considering efficacy, predictability, and influencing factors by comparing it with multibracket therapies. The Boolean keywords: "Crowding" AND "Aligners" were used in PubMed, Scopus, and Web of Science databases with a constraint on English-language papers from 1 January 2013 through 1 August 2023. After removing duplicates examining and verifying the eligibility of the 345 articles that the automated search produced, 16 papers were included. Clear aligners (CAs) are efficient, particularly in cases requiring precise vertical control or correcting single-tooth crossbites by acting as support surfaces; however, challenges such as accurate tracking and managing expansion levels must be considered during treatment planning.

KEYWORDS: *clear aligners, crowding, expansion, crossbite, orthodontic, treatment, anchorage*

INTRODUCTION

The idea of moving teeth without using traditional orthodontic appliances was introduced by Dr. H.D. Kesling in 1945 through a flexible dental positioning appliance (1). Progressively, various techniques were developed, including using invisible aligners and the Raintree Essix technique based on plaster models to correct minor dental discrepancies

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(2). However, these techniques were limited to small displacements of 2-3 mm (3). The introduction of Align Technology's Invisalign™ clear aligner in 1998 revolutionized orthodontics by providing more comfortable and esthetical adult treatments (4, 5). Transparent aligners are a valid alternative for resolving malocclusions compared to fixed appliances, especially when superior vertical control is required (6). Fixed appliances exert tension on the teeth, while clear aligners (CAs) exert thrust, a significant distinction between how a fixed appliance moves teeth and how CAs do (7, 8). In multibracket therapy, the flexibility of the archwire and the amount of deflection required to engage the tooth determine the force applied to the tooth (4, 9). A thicker, stiffer arch provides better arch-apparatus contact in the fixed appliance technique because tip, torque, and in-out information are encoded in the brackets and expressed more fully in an arch with proportions close to those of the bracket slot (10-14). In contrast, when an aligner is worn, slight variations exist between the teeth' positions in the mouth and the aligner's positions (15). Because of the material's flexibility, the aligner conforms to the teeth and pushes them into the appropriate position (16). The improved attachments give the aligner a bigger, flatter, and more active surface to push in order to move teeth in various directions, such as rotation (17, 18). Orthodontic information is expressed by touching the dental crown's surface and the aligner (Fig. 1) (19). Increased material and tooth contact guarantee better expression of the required movements (16, 20, 21). A better contact is made when teeth have long clinical crowns and a larger surface area (22).

In contrast, there is less contact and less tooth movement in teeth with short clinical crowns and a smaller surface area (23). It is feasible to connect anything to the tooth to expand the surface area of the tooth and, hence, the contact area of the aligner to increase the contact of aligners on teeth with reduced morphology. Anchorage can also be well managed with aligners; anchor segments can be defined at the origin and modified at various therapy stages when CAs are used (24). Because anchor teeth can be made immovable at various stages of treatment, CAs offer superior anchorage control (25).

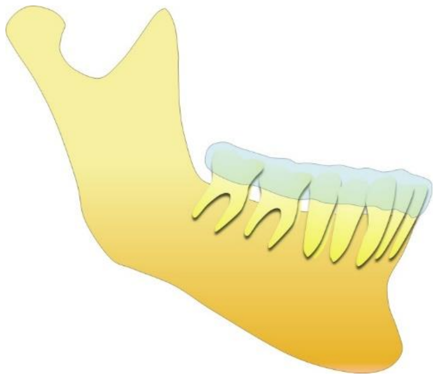

Fig. 1. *Transparent aligner on mandibular arch*.

Dental arch expansion movements are achieved with fixed appliances by buccal tilting of teeth in both posterior and anterior areas (26). With the aligner technique, on the other hand, upper arch development can be planned to use a combination of two tooth movements: buccal tilting of the teeth and translation of the posterior tooth bodies (27). However, several authors agree that more buccal tilting than body translation is observed in practice (28).

The use of aligners for expansion was examined in a study by Haouili et al. (29). The maxillary arch has shifted from a V-shape to a more parabolic shape; premolars have a greater tendency to expand than canines because they are placed on a straight line; canines, on the other hand, move on an arc of a circle whose radius depends on the size of the incisors and canines. According to the study, you can achieve arch growth with CAT using the least vestibular tip (30). Transparent aligners simplify correcting a single tooth's crossbite since they support surfaces that remove occlusal hindrance and resolve the crossbite (31). Multiple teeth in a cross-bite might be more challenging to correct, often by rotating the teeth' crowns under vestibular torque. At the canines, molars, and premolars level, the expansion can be carried out simultaneously or differentiated by stopping one sector at a time (32). In crossbite cases, hypercorrect expansion is advised in the first programming until the mandibular molars' buccal cusps touch the upper molars' palatal cusps. When expansion is greater than 2 mm, transverse elastic bands or other tools may be needed. In addition, it has been shown that the maximum amount of arch width expansion should be 2-3 mm per quadrant to minimize the danger of gingival recurrence and recession. According to one study, the upper first molars have the least accurate tracking. The most plausible causes of this discrepancy are root architecture, cortical bone thickness, and increased chewing and cheek soft tissue. Clincheck's prediction of expansion implies a more bodily movement of the teeth than can be observed clinically, in which the greater movement is given by the change in torque of the dental elements involved.

MATERIALS AND METHODS

Protocol and Registration

This systematic review was carried out according to the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guidelines.

Search Processing

Crowding and aligners were the search terms utilized on the databases (Scopus, Web of Science, and PubMed) to select the papers under evaluation, with the Boolean operators "AND". Only English-language publications from the preceding 10 years (August 2013 – August 2023) were included in the search restrictions (Table I).

Table I. *Database search indicators.*

Article screening Strategy	Database: Scopus, Web of Science, and Pubmed
	Keywords: A “Crowding”; B “Aligners”;
	Boolean variable: “AND“
	Timespan: 2013-2023
	Language: English

Eligibility Criteria

The following criteria were used by the reviewers to choose works for inclusion: 1) Research involving only human participants; 2) clinical studies or case reports; and 3) research involving patients undergoing aligner-based orthodontic treatment. Research including periodontitis, in vitro research, animal studies, systematic reviews, narrative reviews, meta-analyses, and case reports were excluded.

Data Processing

The screening process allowed for the exclusion of any publications that did not fit the themes examined. It was carried out by reading the article titles and abstracts selected in the earlier identification step. After being found to meet the predefined inclusion criteria, the full text of the publications was read. Disagreements among reviewers on the selection of the article were discussed and resolved.

RESULTS

A total of 345 publications were found by doing keyword searches across the Web of Science (47), Scopus (56), and Pubmed (242) databases. A total of 275 items were added after 70 duplicates were removed. 250 of these 275 studies were excluded because they did not meet the predetermined inclusion requirements. For this investigation, 11 publications were chosen after the screening step. In Fig. 2, the findings of each study were summarized. Table II summarizes the study's attributes

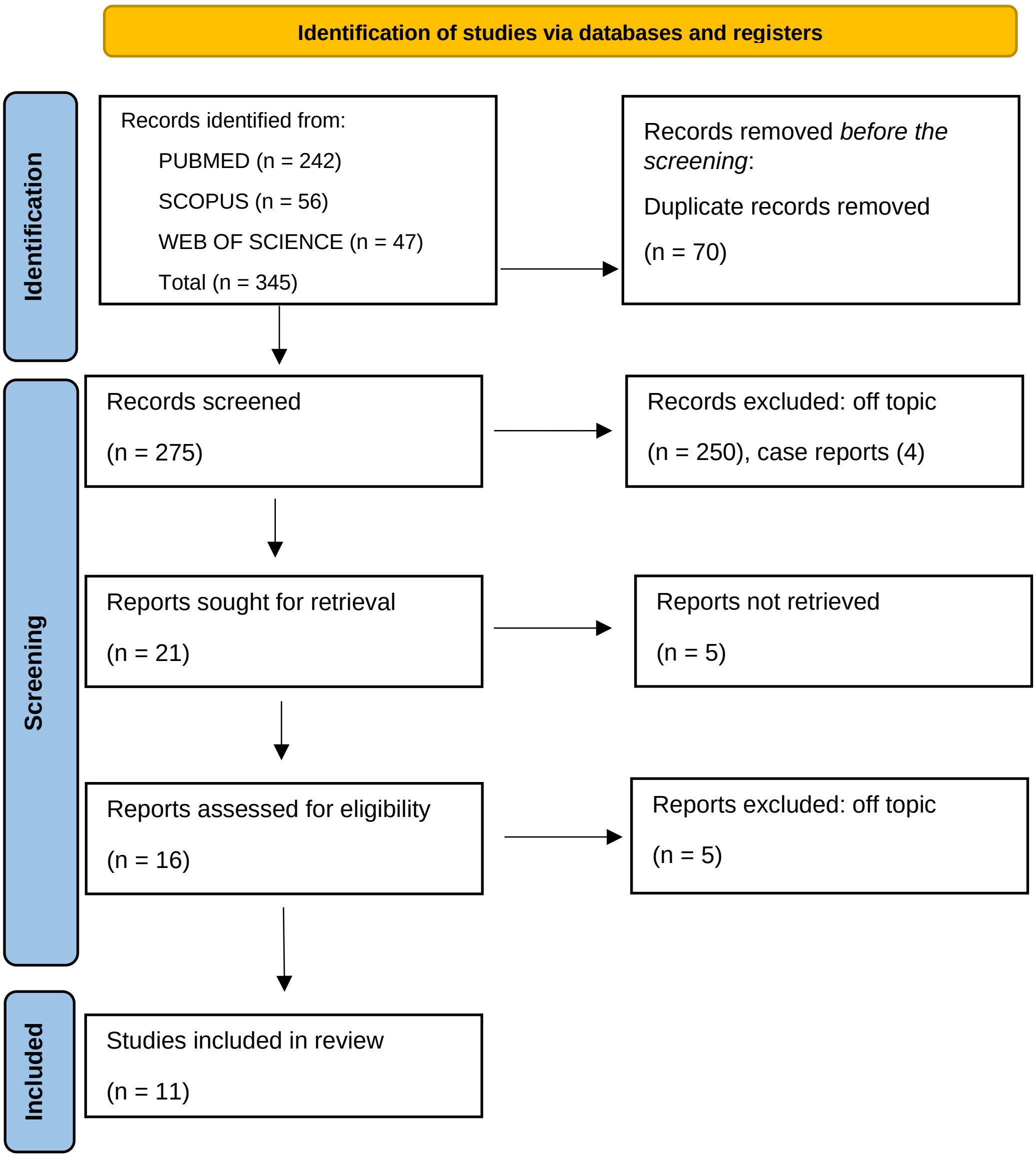

Fig. 2. *Process of inclusion flowchart as seen in PRISMA.*

Table II. *Featured research in the qualitative analysis and their characteristics.*

Authors (Year)	Study Design	Patient's Number/ Gender/ Mean Age	Appliances	Analyzed Parameter	Outcomes
De Felice et al., 2020 (33)	prospective study	40 subjects and 80 dental arches	Doctor Locator by Align Technology (California)	The amount of actual IPR conducted and the amount of planned IPR were recorded for each patient. Each arch was given unique consideration. The second-to-second premolars of each arch's mesiodistal teeth were measured.	The current study compared the accuracy of the real space created by IPR to the amount of IPR intended during CAT using a computerized setup.

Palone et al., 2023 (34)	Clinical retrospective study	150 patients (80 women and 70 men; mean age 33.7 ± 12.7 years)	CAT	Inclination (buccal-lingual crown tipping), angulation (mesial-distal crown tipping), rotation, intrusion, and extrusion	Most adjustments were needed for inclination and rotation, whereas minor corrections were required for angulation, intrusion, and extrusion. Except for extrusion, all corrective movements depended on the tooth type and the recommended movement's size.
Ashutosh et al. 2023 (35)	Prospective clinical research	72 patients, adults of both genders, affected by mild to moderate crowding, divided into 2 groups of 36, one treated with fixed orthodontics, the other with aligners	Ni-Ti wires and CA	Correction of mandibular anterior crowding	Ni-Ti wires and clear aligners worked well for treating cases of mandibular anterior crowding.
Hennessy et al. 2016 (36)	Randomized clinical trial	44 patients (mean age, 26.4 ± 7.7 years) were randomized in a 1:1 ratio to either the fixed labial appliance or the clear aligner group	Fixed labial appliance and the CA group	Proclination of lower incisors because of crowding resolution	In mild crowding cases, there was no difference in the quantity of mandibular incisor proclination brought about by clear aligners and fixed labial appliances.
Jaber et al. 2022 (7)	Randomized clinical trial	36 adult patients (19 females, 17 males; age range: 18 to 25 years) who had severe crowding	CA and fixed appliances	Evaluation of level of oral health-related quality of life (OHRQoL)	During the first year of treatment, patients who use clear aligners experience a lower impact on OHRQoL than those who use traditional fixed appliances.
Jaber et al. 2022 (37)	Randomized clinical trial	36 patients (12 males, 24 females; mean age: 21.24 ± 2.33) with severe crowding	CA and fixed appliances	Effectiveness and efficiency in the management of extractive cases of 4 first premolars	There are no appreciable differences between the fixed application and CA groups.
Izhar et al. 2019 (38)	Clinical	10 cases, Mild anterior crowding	CA	Tooth movement	- Irregularity scores higher in clinical STL group at T4, T6, T8 stages compared to software model group - Mean accuracy: T4 (62.5%), T6 (68.8%), T8 (78.1%) Overestimation by predicted software models compared to actual tooth position -Need for overcorrection in treatment planning and execution

Hellak et al. 2018 (39)	Retrospective	30 patients (28 women, 2 men) / Mean age: 36.03 ± 9.7 years	Invisalign CA + IPR	Interradicular bone volume	- Slight increase in bone volume overall (0.12 ± 0.73 mm) - Highly significant increase in mandible (0.40 ± 0.62 mm; - Slight loss of bone in the maxilla, significant in the apical third (-0.16 ± 0.77 mm; - Positive effect on interradicular bone volume in crowding treatment, particularly in severe cases (periodontally high-risk dentition) - Effect appears independent of IPR
Fiori et al. 2022 (40)	Clinical study	40 subjects	CA and manual stripping	Little's Irregularity Index, Transversal arch diameters (intercuspid, inter premolar, and intermolar width), Incisor position/arch length, Enamel interproximal reduction (IPR)	Predictability of crowding resolution: 87% in the upper arch and 81% in the lower arch. Variations in sagittal incisor position were predictable (70% upper and lower arch). Changes in arch diameters were less reliable (49-67% lower arch, 59-83% upper arch). IPR is least accurate (49% upper arch, 42% lower arch).
Ren et al. 2022 (41)	Observational Study	31 patients (10 males, 20 females; age 14-44)	CA (Invisalign)	Tooth Movements: Maxillary first molars, canines, central incisors	Undesirable tooth movements were observed, influenced by age, crowding, mini-implant, overbite, and attachments. Vertical rectangular attachments on canines were beneficial for achieving more predictable incisor tooth movements over-optimized canine attachments.
D'Antò et al. 2023 (42)	Clinical study	30 adult patients (27 ± 6.1 yo.)	CA	Dentoalveolar expansion and molar inclination	In all cases, except for molar inclination, a statistically significant difference was found between achieved movement and prescription. -Expansion is achieved mainly by tipping the crown rather than bodily movement. -Virtual plan overestimates tooth expansion; overcorrection may be reasonable for highly contracted arches.

DISCUSSION

Given the lack of studies comparing predicted outcomes with clinical outcomes of CA and the consequent need for such comparisons to improve understanding of treatment, the work of Izhar et al. conducted an in-depth analysis of the efficacy and accuracy of CA in modern orthodontics, focusing on comparing predicted software models with actual clinical outcomes of CA. Biological components like age, gender, periodontal ligament, root length, bone density, systemic conditions, and variables that may influence orthodontic tooth displacement were considered (38). A detailed analysis of the results found that software models consistently overestimate tooth alignment and discrepancy resolution compared with actual clinical outcomes at various times during treatment. However, aligner accuracy improves as treatment progresses, reaching about 78% accuracy at time T8. This suggests that aligner effectiveness increases with time, potentially due to patient adaptation mechanisms. The research emphasizes the importance of planning and performing the treatment with the necessary overcorrection to achieve the desired results; in addition, the authors agree with the scientific literature that variables such as the stiffness of the aligner material and proper patient adherence need to be considered for aligner success (34). Thus, the results reveal the complexity of factors involved in achieving accurate results and underscore the importance of further research to refine the application of aligners and optimize clinical outcomes. The work of D'Anto et al. (42) sheds important light on areas of development and the need for rigorous treatment planning and monitoring by revealing the limitations and predictability of dentoalveolar growth with CA.

The findings of a detailed analysis of the predictability and accuracy of dentoalveolar expansion using Ordoline aligners revealed that the average accuracy of upper arch expansion at the cuspidal level was 71%, as opposed to 60% at the gingival level. The lower arch followed a similar pattern, with accuracy scores of 67% at the cuspidal level and 59% at the gingival level. Notably, the degree of expansion varied among teeth, with canines showing more mobility than premolars and molars having the least expansion. The data indicate that aligners essentially produce crown tipping rather than actual tooth body movement, including root displacement, and that there is a significant increase in molar inclination when aligners are used for expansion. Understanding that aligners rarely achieve 100% of the recommended expansion is critical. Consequently, when arranging orthodontic treatment with aligners, clinicians must consider ongoing monitoring and overcorrection of expansion.

Hyper-correction

Palone et al. recently analyzed data from 150 patients successfully treated with CA therapy. Their study provides clinical information on overcorrection to include in initial planning to optimize CA therapy. The expected cases involved Class I malocclusion with minimal crowding. The findings revealed that tilt and rotation required the most correction, while angulation, intrusion, and extrusion required the least. The analysis also showed that the correction amount depended on the type of movement prescribed and the type of tooth. They, therefore, highlighted in this study that, to optimize CA therapy, it is necessary to add approximately 20% overcorrection in the initial planning, especially for complex movements such as inclination and rotation (34).

Interproximal enamel reduction

The quantity of dental enamel removed during CA scans and what was digitally planned were compared by De Felice et al (33). Interproximal enamel reduction (IPR) is a standard procedure in orthodontic treatment with CA, but its accuracy can vary depending on several factors. One of the primary justifications for doing IPR is dental crowding, and the extent of crowding can differ from case to case and can affect the amount of enamel to be removed. The factors related to the IPR procedure are Virtual Digital planning as a process through which the amount of IPR necessary is programmed using specific software based on digital models of the teeth, the operator's experience, which is a key factor in the accuracy of the procedure and the technique chosen which may influence the accuracy of the procedure. The IPR technique mainly uses diamond strips or diamond discs. Forty patients were involved in De Felice's study. The quantity of enamel removed in vivo did not always match the digital planning, although the variances had little clinical significance. The average discrepancy between the digital planning and the IPR was 0.55 mm in the upper arch and 0.82 mm in the lower arch.

Measurement protocol for IPR

1. Mesiodistal tooth measurement considering long tooth axis (Fig. 3).

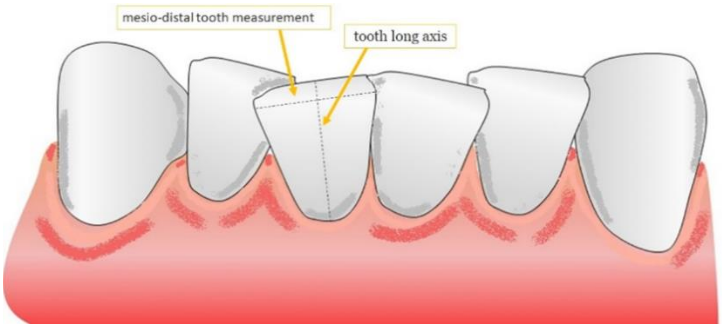

Fig. 3. *Mesiodistal tooth incisor measurement and tooth long incisor axis.*

2. For each arch, the dimensions of the mesiodistal teeth are measured from the second to the second premolar before and after IPR (Fig. 4).

Fig. 4. *Mesio-distal teeth diameters from 35 to 45.*

In the quest for functionally healthy and aesthetically attractive dentition among adult patients, the treatment of crowding and malpositioning of adults in an inconspicuous manner has garnered significant attention. Numerous therapeutic approaches have been investigated to address these issues, including the use of aligners and IPR, reasoning that in his study, Andreas Hellak et al. (39) analyzed the dynamics of orthodontic treatment for crowding in adults.

The study evaluated 720 measurement points to examine treatment effects through pre- and post-therapy cone-beam computed tomography (CBCT) scans of 30 adult patients (28 women, two men). The mean age of the patients was 36.03 ± 9.7 years. The study analyzed interradicular distance changes, focusing on the mandible and maxilla. The results revealed that interradicular distance increased in the mandible while a decrease was observed in the maxilla. The study examined the influence of IPR on interradicular space and observed that remodeling of the dental arches outweighed the effects of IPR on changes in interradicular space. Treatment of crowding had a positive impact on interradicular bone volume, especially in subjects with periodontal risk dentition.

In cases where the initial root distance was less than or equal to 0.8 mm, the treatment improved interradicular spatial conditions. These results highlight the effectiveness of aligner therapy and IPR in alleviating crowding-related problems in adults, particularly in subjects with a high risk of periodontal issues. Still, there is no doubt that further investigation is needed to provide additional insights into this intricate interaction between orthodontic interventions and bone dynamics. The study by Fiori et al. aimed to assess the efficiency of various tactics for gaining space in CAs and the predictability of crowding resolution, as well as their correlation to establish an appropriate protocol to achieve predictable results (40). The sample consisted of 40 adult subjects with severe crowding (mean age 34 ± 7) undergoing non-extractive orthodontic treatment with CA. Following computerized planning, there was still 1.83 mm of residual crowding in the top arch and 1.40 mm in the lower arch. High levels of crowding resolution prediction were observed, with 87% in the upper arch and 81% in the lower arch. Transverse arch expansion showed predictability ranging from 59% to 83% in the upper arch and 49% to 67% in the lower arch, with variability among different tooth positions. The sagittal arch length was moderately predictable, with an accuracy of about 70% in both arches. In terms of IPR, less than half of the planned IPR was achieved, indicating lower accuracy, with 49% predictability in the upper arch and 42% in the lower arch. A strong inverse correlation was observed between increasing intercuspidal diameters (3-3) and crowding resolution. However, the correlation between IPR and crowding resolution was positive but not statistically significant. The study emphasizes the multifactorial nature of predictability, which is influenced by factors such as tooth characteristics, operator experience, and the accuracy of planning software. The results suggest that although CAs effectively solve crowding, they require carefully considering different strategies to gain space. The study emphasizes the importance of the orthodontist's clinical experience in modifying virtual plans and optimizing alignment protocols. Hypercorrection and stepped movements should be considered to improve treatment outcomes. Indeed, further research is needed to incorporate additional measures, such as cephalometric or cone-beam imaging, to improve predictability.

Comparison between fixed orthodontics and aligners

Ashutosh et al. conducted a prospective clinical trial to compare nickel-titanium (NiTi) wires with CA in treating mandibular incisor crowding. Seventy-two participants were randomly divided into two groups, each with 36 samples. Participants in group I received care from NiTi arch wires, while those in group II received care from CA. It can be inferred from the results of the current study that NiTi wires and CA were equally successful in treating cases of mandibular anterior crowding. New in orthodontics, CA may be helpful for patients who are more concerned with aesthetics (35).

Hennessy et al. conducted a randomized clinical experiment to compare the mandibular incisor proclination brought on by fixed labial appliances and CA. Forty-four individuals with minor mandibular incisor crowding (4 mm) received orthodontic treatment using either fixed labial appliances or CA. By comparing lateral cephalograms taken before and after treatment, mandibular incisor proclination was calculated. Mandibular incisor proclination of 5.3° ± 4.3° was caused by fixed appliances. Mandibular incisors in CA were proclinated by 3.4 ± 3.2°. According to the study's findings, there was no statistically significant difference between the two groups (36).

Extractive cases

The purpose of Jaber et al. was to compare the efficacy and efficiency of CA versus conventional fixed appliances in treating complex cases based on premolar extraction. A specific protocol was used in CA case management, and 36 patients were divided into two groups equally and randomly. Three stages of teeth movements were identified in this protocol. In the first stage, the second premolars and molars were used as anchorage units to level and align the overcrowded anterior teeth. The canines were moved first in the initial stage, then the lateral incisors, and finally the central incisors. If necessary, dentoalveolar protrusion of the incisors must be fixed at this stage. If necessary, dentoalveolar protrusion of the incisors must be fixed at this stage. The class I canine and molar relationship was established in the second stage (space closure) by permitting second premolars and molars to move as necessary. The final stage (fine-tuning) involves making necessary adjustments for overcorrection, filling in any residual gaps, and taking final bite alignment measurements using posterior intercuspation methods. Using this protocol, aligners proved effective and efficient in managing extractive cases, and no significant differences were found compared with fixed orthodontic therapy.

Meanwhile, the study results of Ren et al. (41) revealed several important new insights into the predictability and management of tooth movement with CA among patients exposed to premolar extraction. Transparent aligner technologies have advanced significantly over the past two decades, offering advantages such as comfort, aesthetics, and ease of use. These aligners are now recognized for their accuracy in treating a variety of malocclusions, including deep, crooked, open, severe crowding, and scleral anomalies. However, questions remain about the full achievement of orthodontic tooth movements using CA and their variable predictability. Precise biomechanical planning is crucial to ensure successful results, especially in cases involving the removal of premolars and subsequent withdrawal of incisors. These tooth movements are thought to be imperceptible, as demonstrated by the CAT Complexity Assessment Tool (CAT-CAT). The study found that using CA, patients with premolar extraction were able to correct undesirable tooth movements such as lingual overbite, incisor overbite, and mesial overbite of first molars. These actions can cause loss of courage and inappropriate incisor retraction.

Overbite strategies have been suggested to overcome these difficulties and achieve effective exit space closure. However, the optimal amount of overtreatment remains uncertain, underscoring the need for further research. The results show that age, crowding, mini-implants, overbite, overjet, and attachment type all have a distinct role in influencing tooth movements during extraction space closure. Canines are essential to achieve controlled torque and vertical movement of the incisors. In addition, ridges and obstacles influenced how teeth moved, with vertical obstacles on canines demonstrating their value in achieving predictable incisor movements. In conclusion, the study shed light on the challenges and potential solutions related to premolar eviction cases managed by CA. The research aims to improve the predictability and effectiveness of orthodontic tooth movements in these situations by conducting an in-depth analysis of the interactions of many factors. Early images with larger sampling and prosthetic designs are essential to better understand these complex biomechanics and improve treatment outcomes for patients undergoing CA therapy.

OHRQoL = oral health-related quality of life

According to several studies, aligners are as effective as fixed orthodontics, but this cannot be said for oral health-related quality of life (OHRQoL). A randomized clinical trial was carried out by Jaber et al. (7, 37) to assess this index. For the treatment of severe crowding, selected patients were randomly divided into two groups and treated in one with fixed orthodontics and the other with aligners. Before beginning treatment (T0), one week, two weeks, one month, three months, and six months later (T1, T2, T3, and T4, respectively), evaluation questionnaires were given to every patient. The first few months of therapy are when OHRQoL declines the most. OHRQoL gradually improved as treatment was continued.

Patients who used CAs had less effect on OHRQoL during the first year of treatment than those who used conventional fixed appliances (7). Functional limitation, pain, and physical disability had the most detrimental effects on both groups during orthodontic treatment (37).

CONCLUSIONS

The review discusses the effectiveness and challenges of using clear aligners in orthodontic treatment, particularly in crowding and malocclusions. Clear aligners offer a viable alternative to fixed braces, especially when precise vertical control is required. Clear aligners exert a push on the teeth, allowing better control of the anchorage compared to fixed braces that exert tension. However, vestibular inclination is more commonly observed in practice.

Clear aligners are effective in correcting single-tooth crossbites, as they act as support surfaces. However, correcting crossbites involving multiple teeth can be very challenging. Accurately monitoring and managing expansion levels are essential considerations when planning treatment with clear aligners. Clear aligners can achieve high accuracy in resolving crowding, but software models tend to overestimate tooth alignment and resolution of discrepancies compared to actual clinical results. IPR is commonly performed in orthodontic treatment with clear aligners. The accuracy of IPR may vary depending on factors such as operator experience and the technique chosen. Clear aligners are effective in resolving crowding-related issues in adults, particularly those at risk for periodontal problems. However, further research is needed to refine predictability. The article also compares the effectiveness of clear aligners with fixed orthodontic appliances in various scenarios, including cases of mandibular incisor crowding and premolar extraction. All these findings highlight the importance of individualized planning for each patient, to achieve successful orthodontic results.

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Review

CONSEQUENCES OF CARBONATED BEVERAGES ON TOOTH ENAMEL: A SYSTEMATIC REVIEW

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ABSTRACT

The purpose of the current study was to assess the potential erosive effects of rising fizzy drink consumption on tooth surfaces. The study employed a rigorous methodology, using the Boolean keywords "soft drinks" AND "tooth" to conduct a detailed investigation in PubMed, Scopus, and Web of Science during the last five years (2018-2023). Of the initial 407 discovered publications, 18 studies were included, consisting of 12 *in vitro* and 7 *in vivo* research. The findings suggest that overindulgence in acidic carbonated beverages raises the possibility of dental erosion, which results in enamel deterioration and loss of its mechanical and physical qualities. Rougher surfaces, as a result of erosion, encourage more bacterial adherence and a higher possibility of cavities. Most commercial carbonated drinks have a pH lower than what is necessary for the demineralization of enamel. The length of exposure to these beverages and their acidity have different negative effects on enamel.

KEYWORDS: *nutrition, carbonated drink, demineralization, tooth, enamel erosion, dental hypersensitivity, oral pH, tooth decay, soft drinks, bacterial colonization*

INTRODUCTION

One of the most common diseases in both developed and developing countries is tooth decay, which is caused by various socioeconomic, behavioral, genetic, and environmental factors (1, 2). Acids, intrinsic and extrinsic, are among the leading causes of tooth erosion, with the pH of food and beverages playing an important role. However, other factors, such as saliva composition, eating habits, and lifestyle, can influence the erosive process (3-5). Children drank more carbonated drinks (CDs) as a result of the COVID-19 pandemic's alteration in dietary habits. Dental caries rates have increased due to this (6, 7).

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Optimizing public health during this epidemic requires knowledge from social and behavioral studies, lifestyle, and all human sciences relevant to diet and lifestyle, in addition to knowledge from the biological and medical sciences (8).

Several studies on the subject found that the growing habit of drinking CDs, sometimes instead of water, increases the risk of erosion of dental hard tissue (9, 10).

Due to the presence of acids such as carbonic, citric, phosphoric, and tartaric acid, the pH of CD is highly acidic, with values as high as 2.5 (11-14). These acids can erode tooth enamel (Fig. 1, 2), causing pain and hypersensitivity (15). Many factors, including the amount and type of acids in drinks, buffering capacity, temperature, and morphology of tooth enamel, influence the chemical-mechanical process known as tooth erosion (16, 17). In addition, the Faurier infrared spectrum has been used to study the effects of erosion at the chemical level (18-20).

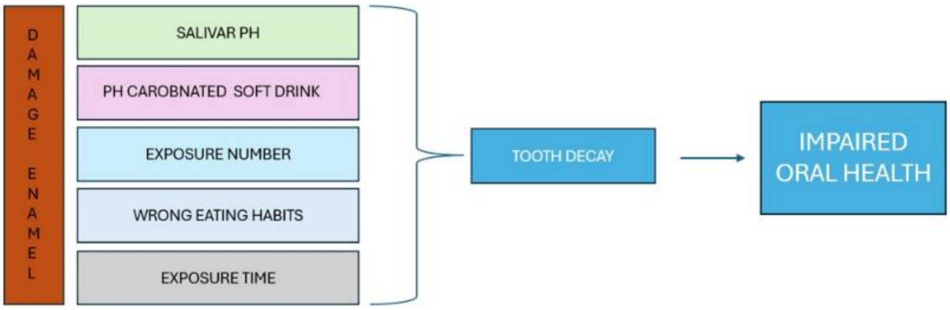

Fig. 1. *The causes of enamel damage.*

Fig. 2. *Example of the consequences of carbonated beverages on tooth enamel.*

MATERIALS AND METHODS

Registration and protocol

The current systematic review followed the PRISMA and International Prospective Register of Systematic Review Registry procedures (full ID:549705). The following databases, PubMed, Web of Science (WOS), and Scopus, were examined from January 1, 2018, to November 11, 2023, to search articles of the last 6 years. The search strategy was created by combining terms relevant to the study's purpose (Fig. 3).

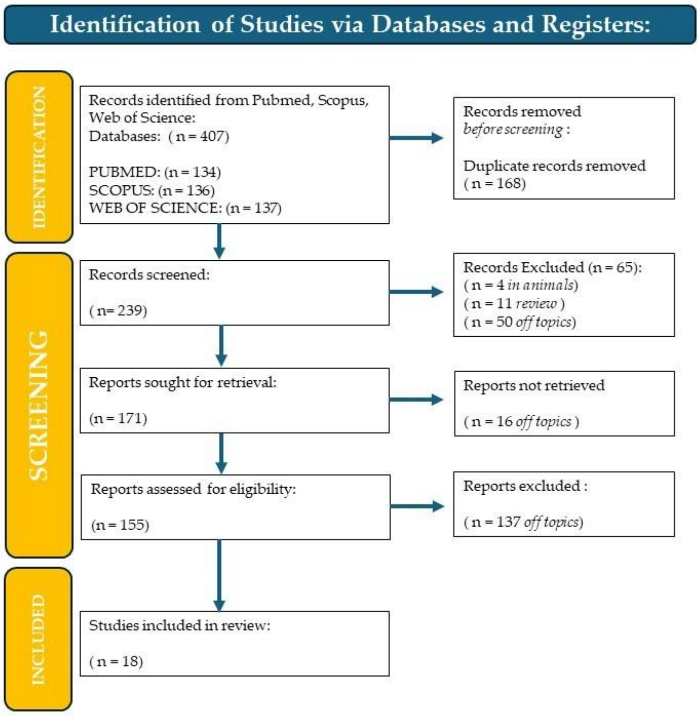

Fig. 3. *PRISMA flowchart.*

The process of searching

Scopus, PubMed, and WOS were searched with an English language restriction to find papers that fit the topics published between January 1, 2018, and November 11, 2023. Because they were a combination of words that matched the objective of our analysis, the following Boolean keywords were added to the search strategy: “teeth” AND “soft drinks”.

Eligibility criteria

Inclusion criteria:
(1) human subjects' *in vivo* and *in vitro* studies;
(2) linguistic studies;
(3) open-access studies;
(4) randomized clinical trials;
(5) studies on the impact of carbonated beverages on dentin enamel comprise the remaining body of research.
Exclusion criteria:
(1) research involving animals;
(2) research conducted in languages other than English;
(3) research with restricted access;
(4) case studies, series, reviews, editorials, and book chapters.
The PICOS criteria were used to conduct the review:
• Participants: adult and pediatric teeth, both *in vivo* and *in vitro*, were used.
• Steps taken: consuming a lot of fizzy beverages.
• Comparisons: CDs are not used very often.
• The outcome is damaged dental enamel.
Research: human teeth subjected to *in vitro* and *in vivo* clinical testing.

Risk of bias measurement

RevMan 5.5 software was used to evaluate the risk of bias in the selected studies. The evaluation followed the Oral Health Assessment Tool (OHAT) criteria, which covered aspects like performance and detection blinding, partial reporting, allocation concealment, and randomization. Seven *in vivo* studies were analyzed to determine the risk of bias.

The risks were divided into three categories: adequate, ambiguous, and inadequate. Studies were considered to have a low risk of bias if they met at least five to seven positive criteria without adverse outcomes. *In vitro* studies were excluded from the report (Fig. 4.)

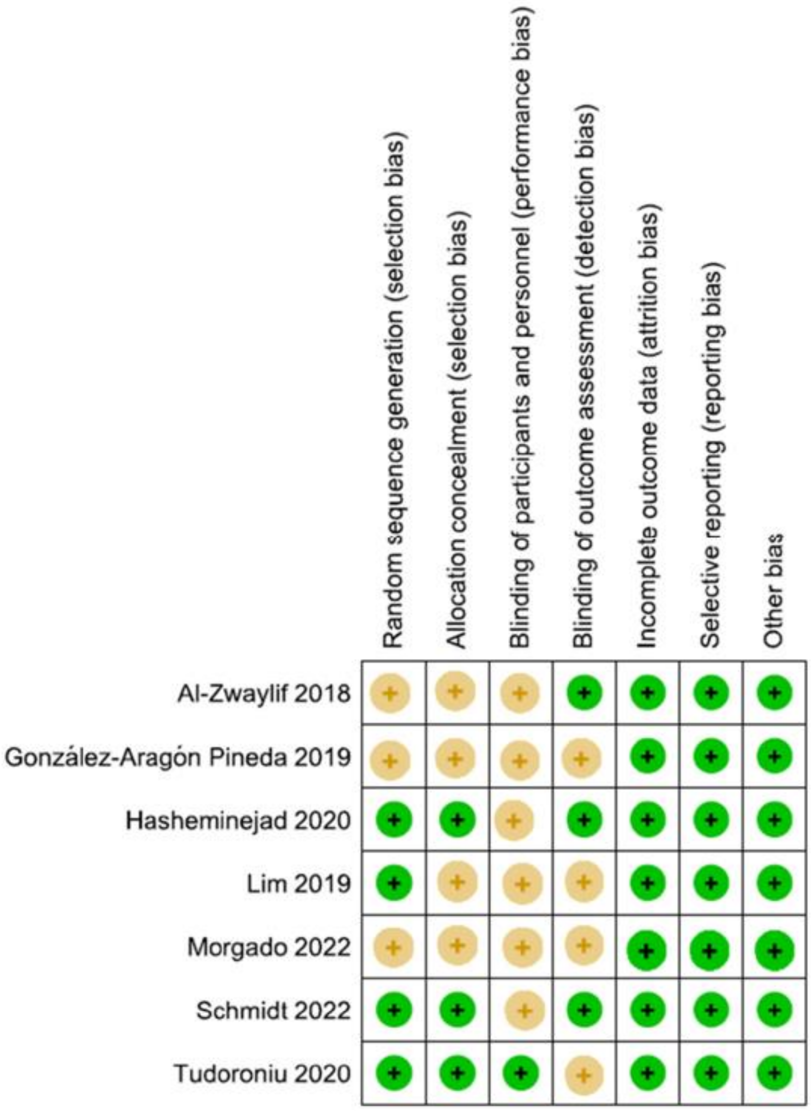

Fig. 4. *Bias risk graphs.*

RESULTS

Four hundred and seven articles (PubMed n = 134, Scopus n = 136, and WOS n = 137) remained from the original search after 168 duplicates were removed. Therefore, 239 articles remained. A total of 156 articles advanced to the screening phase; however, 83 were disqualified for the reasons listed below: 11 were reviews, 16 had no free complete text, 3 had animal-related content, and 53 were off-topic. Nineteen recordings, 12 *in vitro* and 7 *in vivo* were eventually included in the inclusion phase after an additional 137 articles were removed from these products since the supplied data did not pique their attention. The results of each experiment are displayed in Table I (21-36).

Table I. *Descriptive summary of in vitro and in vivo investigations.*

Authors (years)	Type of Study	Aim of study	Materials	Results
Gotouda et al. (2017) (21)	Study *in vitro*	Examine how different beverages and enamel respond to CD.	X-ray microdiffraction examination demonstrated varying levels of enamel white spots, ranging from slight to significant deterioration.	Important crystallographic information from this study will support preventive dentistry.
Shroff et al. (2018) (22)	Study *in vitro*	Assess the potential erosive impact of 20 drinks.	Six milliliters of fluid had to be dispensed. Saliva was utilized to prepare enamel samples, and 5 g or 7 g of the experimental beverages were used to measure their acidity. Each drink's cumulative weight reduction was calculated across various time intervals.	The weight loss of the experimental beverages significantly differed from that of the packaged fruit juices after 24-hour immersion in CD due to their higher acidity values.
Al-Zwaylif et al. (2018) (23)	*In vivo* study	Examine the connection between tooth wear and dietary acid consumption and timing.	Three thousand five hundred and eighty-six individuals took part. Information was acquired regarding the four types of acidic meals, when they should be consumed, how tooth wear affects the surface area of teeth, and how to consume acidic foods.	Regular consumption of soft drinks is associated with tooth erosion. Soft beverages and meals are linked to moderate to severe deterioration. Other acidic foods are not connected to the decline.
González-Aragón Pined et al. (2019) (24)	*In vivo* cross-sectional study	Examine the connection between various beverages and erosive toothwear (ETW).	A questionnaire will be used to monitor the frequency of consumption of various beverages such as milk, water, fruit juices, hot beverages, and soft drinks.	To avoid ETW, milk and milk products could be a decent substitute for sugary beverages.
Lim et al. (2019) (25)	*In vivo* study	Analyze the long-term impact of soda consumption on children's dental cavities.	Nine hundred ninety-five pairs of carer and child. Keeping an eye on kids' dental surfaces was the task. Caregiver smoking, oral health fatalism, and social support were among the variables.	Those who drank a lot of soda had a higher rate of cavities than those who didn't. The work emphasizes how modeling problems in longitudinal research can be addressed with focused maximum likelihood estimates.
Charpe et al. (2019) (26)	*In vitro* study	Examine the differences in dental enamel's solubility following different durations of alcohol exposure.	Using extracted teeth, three distinct beverages were examined for their ability to dissolve enamel at various intervals. The Calcium Reagent Set and a semi-automatic analyzer were used to analyze calcium release.	Significant calcium loss was seen in relation to beer, whiskey, soft drinks, and hard beverages.
Kono et al. (2019)(27)	*In vivo* study	Teeth sections were subjected to micro-FTIR spectroscopy to elucidate the chemical mechanisms involved in dental caries.	According to X-ray microdiffraction research, the enamel contained a wide range of white stain areas, from almost undetectable to almost completely decayed.	This study demonstrated the range and normalcy of tooth enamel properties.
Panic et al. (2019) (28)	*In vitro* study	Find out how CD affected dentin and enamel over time.	Twenty samples were evaluated and photographed using SEM at 60 minutes, 24 hours, and 7 days after exposure to drinks. The results were examined using ANOVA.	The drinks' pH values were lower than the enamel's critical pH, and degradation started to show after just 60 minutes of exposure.
Paula et al. (2019) (29)	*In vitro* study	Juices' pH, acidity, and erosive potential are assessed.	After being surgically extracted, fifteen third molars were immersed in a juice and citric acid solution for four days. The microhardness and roughness of the samples were measured both before and after the erosive cycles. To examine variance, ANOVA was employed.	Juices can be erosive due to their acidic pH.
Ramya et al. (2020) (30)	*In vitro* study	Examine the impact of soft drinks on the demineralization of extracted teeth.	Teeth that have been removed. Their mass was ascertained using a computerized balance. Ten teeth were inserted into every soft drink serving over a specific period. Again, their weight was analyzed. The weight shift was observed, and the outcomes were explained. The study found that the teeth exposed to fizzy beverages lost weight due to the chemicals in the drinks degrading their mineral makeup.	The effects of CD and non-CD on tooth structure are not the same.

Hasheminejad et al. (2020) (31)	*In vivo* study	Examine the relationship between drinking habits, tooth erosion, and dental caries.	Survey on the usual drinking patterns of 600 teenagers.	Teenagers often drink dangerous drinks. Soft drinks have been related to increased erosion and caries, but milk has been demonstrated to protect against caries.
Chandrasekhar et al. (2020) (32)	*In vitro* study	Compare the surface microhardness and mineral loss of enamel exposed to CD 1 with and without calcium glycerophosphate (CaGP).	The surface microhardness of forty enamel samples was evaluated to quantify mineral loss using spectrophotometric analysis and four cycles of intermittent blotting.	The enamel's mineral loss and deterioration in surface microhardness were significantly halted by adding CaGP to the CD.
Tudoroniu et al. (2020) (10)	*In vivo* study	Assess the incidence of dental caries in teenagers and examine the connection between sugary food intake and dental care habits.	A survey of 650 teenagers examined the relationship between the DMFT index, dental cleanliness, and eating behaviors.	Teenagers still have a high prevalence of caries, influenced by their dietary patterns.
Al-Amri et al. (2021) (33)	*In vitro* study	The enamel is subjected to minute alterations as a result of the beverages' other contents, pH, and duration of display.	Three sets of removed teeth were submerged in sweet beverages and saliva, and changes in tooth surface roughness were measured using a profilometer.	Teeth's surface roughness increased after being exposed to sugary beverages.
Sooksompien et al. (2022) (34)	*In vitro* study	This study evaluated morphological and atomic percentage (at.%) changes in primary teeth's enamel surfaces after they were exposed to carbonated soft drinks using energy dispersive spectroscopy (EDS) and scanning electron microscopy (SEM).	1. Children had the extraction of their 45 first molars; 2. they were immersed in commercial soft drinks or deionized water; 3. the enamel surface underwent morphological changes.	The acidic pH of soft drinks changed the enamel's surface.
Arafa et al. (2022) (35)	*In vitro* study	Carbonated soft drinks cause reactions in dental dentin and enamel.	Teeth displayed a wide spectrum of enamel deterioration, both microscopic and by X-ray microdiffraction studies following a week of exposure to fizzy drinks.	Compared to saliva, milk did not exhibit any erosive effects on the enamel surface, while soft beverages did.
Schmidt et al. (2022) (36)	*In vivo* study	By measuring the awareness of dental erosion, one can ascertain the relationships between sociodemographic traits, awareness and knowledge of tooth erosion, and beverage drinking patterns.	Four hundred eighteen students completed an online survey.	Fruit juices and soft drinks were the most commonly consumed acidic beverages. Students who were aware of the risk of tooth erosion drank fewer acidic beverages regularly. The majority of overseas students had less knowledge regarding dental degeneration. Older students studying health-related subjects correctly identified a greater number of varieties of acidic beverages.
Morgado et al. (2022) (2)	*In vivo* study	Educate patients and healthcare providers about the erosive potential of bottled water and analyze its pH levels to address the growing problem of dental erosion, particularly in high-risk populations.	pH analysis of 105 bottled water samples: -32 were carbonated; -73 were non-carbonated.	Bottled water in Portugal varies in pH values. Some brands have pH values below the critical threshold for enamel and dentin, indicating a higher risk of dental erosion based solely on the pH parameter.

DISCUSSION

The article focuses on the harmful effect of CDs on enamel. CDs are becoming increasingly popular in contemporary society and can replace water as the main beverage; however, these types of drinks expose teeth to the acids that are present in these drinks (2, 21, 36). Citric, phosphoric, and carbonic acids lower the oral pH, promoting enamel erosion(10, 37, 38).

Although dental enamel is the hardest part of the human body, the chemicals in CD can affect it (33, 39, 40). The hardness and roughness of the enamel surface are evaluated, and parameters show how severe the damage caused by the acids in CD is (22, 41, 42). Research indicates that consuming these drinks on a daily basis dramatically raises the risk of tooth erosion (35, 43).

Dental erosion is a non-carious lesion that results in the tooth's surface losing its dentine and enamel permanently (44). Various internal and external causes influence this illness, but lifestyle and food choices play a significant role. Younger age groups are more likely to experience dental issues (23, 24, 34).

According to epidemiological studies, CD and fruit juices are among the most acidic beverages consumed by children and young people (14, 25, 45). Dental erosion is increased by the increased consumption of acidic drinks, which is more common in young people (46). However, data on the prevalence of dental erosion show a tendency to be more severe in younger groups (27, 46).

Socio-economic consequences are evident, as people with a higher socioeconomic status will likely have better oral hygiene and more frequent dental check-ups (47). Furthermore, regular drinking of carbonated beverages has been shown to increase the risk of dental erosion, while those who avoid drinking these beverages have a significantly lower risk (28, 29).

Due to the different structural compositions of enamel and dentine, the erosive effects of CD vary. Dentin and enamel leak more quickly, but both are sensitive to the acids in CD. To avoid dental erosion, it is important to understand how regular consumption of CDs affects this problem. Tooth erosion can increase sensitivity and enamel wear, which can have a negative impact on overall dental health (10, 30, 32)

Studies highlight the significance of raising cognizance of the harmful impacts of CD and implementing proactive steps to safeguard oral health, particularly among youth. Promoting good oral hygiene, forbidding the consumption of CDs, and promoting the use of water and other wholesome substitutes (31).

Saliva normally increases in response to drinking and protects dental enamel from acidic beverages. However, individuals with reduced salivary flow are likelier to have eroded enamel. Dental erosion occurs when enamel components such as fluorapatite and enamel hydroxyapatite are exposed to a significantly lower pH than these substances, i.e., between 4.3 and 4.5 (33, 35).

Research has examined the erosive potential of CD and fruit juices based on their pH, mineral content, and capacity to chelate calcium from food and beverages. Research indicates that consuming CD regularly is associated with a higher risk of tooth degeneration, with a greater degree of severity for frequent consumers (48). On the other hand, phosphate, calcium, and fluoride added to beverages can decrease erosion and improve enamel solubility (10, 11, 27).
Saliva removes acids from the tooth surface and acts as a protective barrier, which prevents erosion (17, 49, 50).

Although these tests did not simulate natural mouth conditions, they evaluated erosion on dental samples immersed in acidic beverages (22). However, mineral loss was significantly reduced when saliva was used to preserve dental samples (29, 34, 36).

In conclusion, preventing dental erosion brought on by acidic beverages requires an awareness of the function of saliva and restricting the consumption of carbonated beverages. Dental erosion can result in discomfort and hypersensitivity. Its health status can be determined by evaluating the enamel surface to determine the erosion risk (2, 25).

The increasing consumption of fizzy drinks among young people increases the likelihood of tooth decay and erosion(27, 51). Using X-ray microdiffractometry, studies examined how dentine and enamel reacted to CD. Incorporating buffering agents such as CaGP in carbonated beverages can reduce erosion, but the taste of the beverages prevents this (21, 27, 35). The risk of tooth damage is related to consumption during meals. Sports such as rugby increase the likelihood of erosion. Sports and fizzy drinks make enamel less hard. In contrast, milk reduces the formation of caries (24, 35).

CONCLUSIONS

Consumption of carbonated beverages has increased, raising concerns about oral health. The public should be actively educated about tooth erosion by dentists and medical professionals and encouraged to follow healthier lifestyles.

The quality of tooth enamel depends on the pH of saliva. People with little saliva and frequently drink acidic beverages are more vulnerable to enamel erosion. The first tip is to drink water and include fresh fruit in a balanced diet.

Healthcare providers must persuade individuals to abstain from acidic beverages, and policymakers should suggest ways to reduce sugar-filled beverages. There are numerous programs available to enhance general and dental health.

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Review

A SYSTEMATIC REVIEW OF TREATMENT METHODS FOR MOLAR/INCISOR HYPOMINERALIZATION

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ABSTRACT

This systematic review aimed to identify relevant studies and their characteristics while thoroughly evaluating the literature on treating molar incisor hypomineralization (MIH) or enamel hypomineralization published between 2012 and 2022. Using a precise keyword strategy "(molar incisor hypomineralization) OR (enamel hypomineralization)) AND (treatment)", the search method covered credible academic resources such as PubMed, Scopus, Cochrane Library, and Web of Science. After 637 papers were retrieved, a rigorous selection procedure that complied with PRISMA principles was conducted. Randomized Control Trials (RCTs), case series with more than five clinical cases (CSs), human participant research, full-text articles available for free or via university login, and English-language publications were all included in the inclusion criteria. Systematic or literature reviews, editorials, single-case reports, in vitro experiments, animal studies, paid articles, and publications published in languages other than English were among the exclusion criteria. After a careful screening procedure, 20 articles that the search produced satisfied the strict inclusion criteria. These investigations will form the foundation of an extensive examination of MIH therapy modalities. For a thorough evaluation of MIH treatment approaches, the systematic review guarantees the caliber and applicability of the selected studies. By offering important insights into the features of particular studies, patient profiles, and available treatment options for molar incisor hypomineralization, this systematic review will help improve our understanding of the management of this dental problem.

KEYWORDS: *children, sealants, pediatric dentistry, fluoride, restorative therapies, molar incisor hypomineralization, enamel hypomineralization, treatment, enamel defect, therapy*

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INTRODUCTION

First identified in 2001, molar incisor hypomineralization (MIH) is a developmental disease affecting dental enamel (1, 2). It usually affects the incisors and affects one to four first permanent molars (FPM). MIH has a complicated and multifaceted etiology that includes prenatal and early childhood variables such as infections, fever episodes, neonatal traumas, some childhood illnesses, exposure to environmental chemicals, use of medications during pregnancy or childhood, and difficulties giving birth (3, 4). Despite intensive research, no causative factor has been found (5-7).

In terms of clinical presentation, MIH is characterized by opaque white, yellow, or brown enamel opacities; darker opacities are more likely to experience post-eruptive breakdown (PEB) (8, 9). Because of the enamel's weakened integrity, it is more brittle and prone to breaking (10-12).

Patients frequently have increased dental sensitivity, especially to hot or sweet stimuli. This can have an impact on the appearance and functionality of the teeth, as well as have psychological repercussions on self-esteem (13, 14).

The European Association of Paediatric Dentistry recommends using the modified DDE index, the most often used criterion, for diagnosing MIH. Other indices used in the diagnostic process include the modified DDE index and developing enamel defects (DED) (15, 16). Based on the degree of structural loss and enamel discoloration, MIH severity is categorized into mild, moderate, and severe variants (17, 18). Accurately diagnosing and treating MIH requires distinguishing it from other disorders, including amelogenesis imperfecta and dental fluorosis (19, 20).

Preventive interventions for MIH include regular fluoride treatments and adopting dietary habits that preserve enamel, such as reducing the consumption of acidic and sugary foods (21, 22). Depending on the severity, many treatment options are available: adhesive fillings and crowns are utilized in more severe cases while desensitizing agents and sealants are used in milder situations (23, 24). The goals are to lessen sensitivity, protect enamel, and improve attractiveness (25, 26).

Due to regional and population variations, the prevalence of MIH in children aged 7-13 varies greatly, ranging from 2.4% to 40% (27, 28). More studies are necessary to understand the genesis of MIH, develop diagnostic standards, and advance therapeutic and preventive measures (29, 30) (Fig. 1).

Fig. 1. *Examples of clinical manifestation of incisor affected by MIH.*

MATERIALS AND METHODS

The goal of this systematic review was to compile and evaluate research on MIH therapy that has been published between 2012 and 2022. Using the terms "(molar incisor hypomineralization) OR (enamel hypomineralization) AND (treatment)," a thorough search was carried out across several databases, including PubMed, Scopus, Cochrane Library, and Web of Science. With this search approach, 637 articles were found. Twenty-three publications that satisfied the inclusion criteria were found using a systematic selection procedure that complied with PRISMA principles (31) (Fig. 2).

The criteria for inclusion included research with human subjects, case series with more than five clinical cases, randomized controlled trials (RCTs), randomized controlled clinical trials (RCCTs), and papers written in English. In vitro research, animal studies, editorials, systematic reviews, literature reviews, single-case reports, case series with fewer than five cases, and non-English publications were among the exclusion criteria. It has been registered on PROSPERO with an ID 552859.

A thorough examination of MIH treatment strategies will be built upon the chosen papers. This review aims to give physicians a current overview of the most effective treatments for MIH to improve patient care outcomes and their comprehension of therapy alternatives.

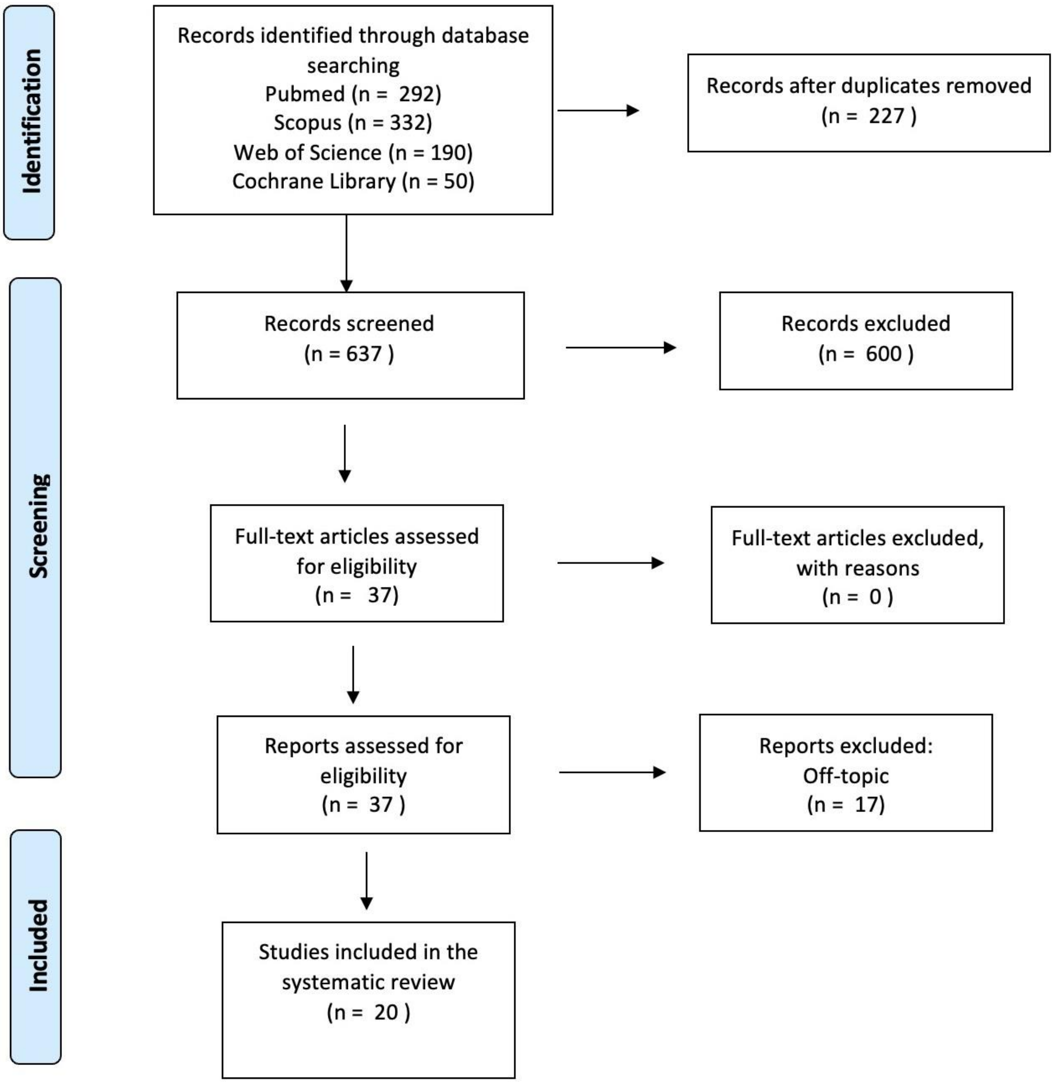

Fig. 3. *PRISMA flowchart diagram of the inclusion process.*

RESULTS

The objective of the systematic review was to examine various techniques for treating MIH. Eighty-six publications were found after thoroughly searching four main databases: PubMed, Scopus, Cochrane Library, and Web of Science. Six hundred thirty-seven items remained after 227 duplicates were eliminated. After a comprehensive screening process that included an analysis of the abstracts and titles, 601 publications that didn't fit the predetermined inclusion criteria were eliminated. After a more thorough eligibility assessment of the remaining 37 articles, 17 irrelevant publications were excluded. As a result, the final qualitative analysis contained 20 studies. These selected papers, which provide a comprehensive assessment of the most recent MIH treatment modalities, serve as the foundation for the review. Table I, which summarizes the results, highlights the most relevant studies included in the review.

Table I. *Summary of the results.*

Author (Year)	Study design	Number of patients	Average age	Gender	Severity of MIH	Treatment	Outcome
Al-Batayneh et al. (2022) (32)	RCT	50	11 years old	26 females, 24 males	Moderate and severe	The study assessed the efficacy of cervical pulpotomy, partial pulpotomy, and indirect pulp therapy.	The study analyzed three VPT procedures: CP (82%) and IPT (96%), and there were no appreciable variations in the success rates.
Ballikaya et al. (2022) (33)	RCT	48	8.8 years old	18 females, 30 males	Mild and moderate	Silver diamine fluoride (SDF) alone versus SDF with glass ionomer cement restoration was found in the study that examined SDF and silver-modified atraumatic restorative treatment (SMART) for molars damaged by MIH.	The study compared SDF and SMART for MIH-affected molars. Both desensitized and prevented cavities; SMART caused marginal discoloration.

Biondi et al. (2017) (34)	RCT	55	6-17 years old	n.d.	Mild with loss of structure	The study applied various treatments for children: sodium fluoride varnish, casein phosphopeptide-amorphous calcium phosphate, and TCP-containing fluoride varnish, assessing their effectiveness over three years.	DIAGNOdent evaluated mineral density in 92 MIH teeth. Duraphat® performed best in moderate lesions and Clinpro® in mild lesions.
De Souza et al. (2017) (35)	RCT	26	6-8 years old	14 females, 12 males	n.d.	Permanent first molars (PMFs) received direct composite restorations, divided into total-etching adhesive (TEA) and self-etching adhesive (SEA) groups, evaluated over 18 months for MIH impact.	SEA and TEA showed high success rates in repairing MIH-affected molars, especially with conservative cavity preparation techniques.
Fragelli et al. (2015) (36)	RCT	21	7.7 years old	12 males, 9 females	Moderate to severe	Glass ionomer cement (GIC) restored MIH-affected teeth. After a year, cautious restoration preserved some MIH-affected areas.	Single-surface restorations had a 78% success rate after one year; delay invasive treatment when possible.
Gatón-Hernandez et al. (2020) (37)	RCT	326 patients	6-8 years old	172 males, 154 females	Severe	Therapy included selective carious tissue excision, GIC restoration, resin composite, fluoride, nutrition counseling, and oral hygiene guidance.	The study examined success rates, sensitivity, restoration integrity, radiographic changes, and apexogenesis as clinical outcomes.
Linner et al. (2020) (38)	RCT	52	11.2 years old	26 males, 26 females	Different degrees	The investigation analyzed CAD/CAM ceramic, conventional composite, non-invasive composite, and GIC restoration procedures.	After 36 months, CAD/CAM ceramic had 100.0% success, conventional composite 76.2%, non-invasive composite 29.9%, and GIC 7.0%.

Luppieri et al. (2022) (39)	RCT	15	n.d.	n.d.	Severe	The study used profilometric and SEM analysis to assess resin infiltration effects on PFM surfaces over time.	Resin infiltration initially smoothed surfaces but later showed increased roughness; hypersensitivity improved and remained constant.
Mendonça et al. (2022) (40)	RCT	60	8 years old	n.d.	Mild and moderate	Three treatments: Experimental Group I (4% titanium tetrafluoride), Experimental Group II (Surface Pre-Reacted Glass Ionomer Filler-containing Coating Resin), and control (sodium fluoride).	Sensitivity was assessed using Schiff, Wong-Baker FACES, and FLACC at intervals; satisfaction was a secondary outcome.
Muniz et al. (2019) (41)	RCT	66	8.89 years old	31 males, 35 females	n.d.	Three treatment groups: (A) low-level laser therapy (LLT), (B) fluoride varnish, and (C) Laser + Fluoride Varnish (L + FV).	Fluoride varnish and combined treatment (L + FV) demonstrated stronger desensitizing effects for MIH-affected teeth.
Murri Dello Diago and Patricia Gatón-Hernandez et al. (2021) (42)	RCT	67	6 to 14 years old	n.d.	EAPD guidelines for MIH in 2010	Infiltration with ICON	Improvements in sensitivity, plaque buildup, and gingivitis
Nogueira et al. (2021) (43)	RCT	51	8.1 years old	25 males, 26 females	Mild	FV was applied four times, fluoride varnish with phosphoric acid pre-treatment (FV + etch) four times, and one resin infiltration treatment.	After 18 months, failure rates were FV 17.9%, FV + etch 17.3%, and resin infiltration 6.10%; molar failures were common.
Olgen et al. (2022) (44)	RCT	49	6-9 years old	23 females, 26 males	Mild and moderate	Group 1: 5% sodium fluoride varnish. Group 2: CPP-ACP. Control: Dental hygiene training.	Remineralization agents improved rates for mild and moderate MIH; ineffective for severe cases.
Özgür et al. (2022) (45)	RCT	39	8.6 ± 1.4 years old	13 females, 26 males	Mild and moderate	Group 1: resin sealant Group 2: giomer sealant	Giomer sealants with self-etch primer fared poorly vs. resin-based sealants over 12 months; lesion size/color didn't affect survival.
Restrepo et al. (2016) (46)	RCT	51	9-12 years old	35 males, 17 females	All degrees	Study group: Four 5% NaF varnish treatments spaced one week apart. Control Group: Typical home health care.	Fluoride varnish did not significantly impact lesion area or fluorescence levels in MIH lesions over time.

Rolim et al. (2021) (47)	RCT	35	10 years old	19 males, 16 females	Severe	FPMs were randomized into TE and SE groups and restored with bulk-fill resin composites and universal adhesive.	The study evaluated restoration survival using updated USPHS criteria. Pain scale and dental anxiety measured before and after treatment.
Sezer et al. (2022) (21)	RCT	53	8-12 years old	28 females, 25 males	Mild and moderate	The study compared calcium glycerophosphate, casein phosphopeptide amorphous calcium fluoride phosphate, and Colgate Toothpaste for dental care on 27, 16, and 10 kids, respectively.	Remineralization was assessed with laser fluorescence (DIAGNOdentTM Pen). Significant differences over time and between groups ($p < 0.001$).
Sobral et al. (2021) (1)	RCT	140	26.5 years old	n.d.	n.d.	The study includes four treatment groups: LLL, PermaSeal, PermaSeal with LLL, and combined LLL and PermaSeal.	Changes in pain/sensitivity measured by VAS at 1 week, 1, 3, and 6 months post-therapy.
Sonmez et al. (2017) (48)	RCT	95 MIH	8.88 years old	n.d.	Moderate, severe	Group I: Completely hypomineralized tissue. Group II: Partially hypomineralized tissue removed. Group III: Deproteinization and partial extraction. Group IV: Control (no MIH).	Group I: 93.7% retention. Group II: 80.7% retention. Group III: 93.5% retention. Group IV: 100% retention. Deproteinization increased retention rates in hypomineralized enamel.
Vicioni-Marques et al. (2022) (49)	RCT	23	8.4 years old	15 males, 8 females	Moderate	Kids received a placebo or ibuprofen before restorative dental care.	The study assessed preventive analgesia's efficacy in reducing hypersensitivity in MIH-affected molars with PEB during and after therapy.

Evaluation of quality and bias risk

Table II reports the risk of bias in the included studies (50). Confounding bias is a major source of bias in most studies. One parameter that has a low chance of bias is measurement bias. Due to participant selection bias, many studies have a low risk of bias. The significant heterogeneity makes it impossible to calculate bias resulting from post-exposure. Many studies have low bias rates because of missing data. In most studies, there is a significant bias in the selection of the published results. According to the final results, ten studies have a high risk of bias, three have a very high risk of bias, and eight have a low risk of bias.

Table II. *Risk of bias.*

Authors (year)	D1	D2	D3	D4	D5	D6	D7	Overall
Al-Batayneh et al. (2022) (32)	X	+	X	-	+	X	+	+
Ballikaya et al. (2022) (33)	X	+	X	+	+	X	+	+
Biondi et al. (2017) (34)	-	-	+	+	-	-	+	-
De Souza et al. (2017) (35)	-	-	-	+	-	-	+	-
Fragelli et al. (2015) (36)	-	+	+	-	+	-	-	-
Gatón-Hernandez et al. (2020) (37)	-	X	+	-	-	-	-	X
Linner et al. (2020) (38)	-	+	X	X	+	-	+	X
Luppieri et al. (2022) (39)	X	+	+	X	-	+	X	X
Mendonça et al. (2022) (40)	-	X	+	-	-	-	-	X
Muniz et al. (2019) (41)	X	+	+	X	-	+	X	X
Murri Dello Diago and Patricia Gatón-Hernandez et al. (2021) (42)	X	-	+	X	+	+	-	X
Nogueira et al. (2021) (43)	X	+	-	-	-	+	-	X
Olgen et al. (2022) (44)	-	+	X	X	+	-	+	X
Özgür et al. (2022) (45)	X	-	+	X	+	+	-	X
Restrepo et al. (2016) (46)	-	!	!	X	-	-	X	!
Rolim et al. (2021) (47)	X	+	+	-	+	X	+	+
Sezer et al. (2022) (21)	X	+	-	-	-	+	-	X
Sobral et al. (2021) (1)	-	+	X	+	-	X	+	+
Sonmez et al. (2017) (48)	?	-	+	!	+	+	X	!
Vicioni-Marques et al. (2022) (49)	X	+	+	-	+	X	+	+

Domains: D1: Bias due to confounding; D2: Bias arising from measurement of the exposure; D3: Bias in selection of participants into the study (or into the analysis); D4: Bias due to post-exposure interventions; D5: Bias due to missing data; D6: Bias arising from measurement of the outcome. ! *Very High;* X *High;* - *some concern;* + *low;* ? *No information.*

DISCUSSION

Treatment for pulpotomy and dental hypersensitivity

When molar incisor hypomineralization (MIH) occurs, dental hypersensitivity causes severe discomfort that affects daily activities, including eating and oral hygiene (Fig. 2). MIH's clinical implications and prevalence are described by Alberto Murri Dello Diago et al. (42), who also promote the use of minimally invasive therapies. Trials are conducted by Fernanda L. Mendonça et al. (40) to create sensitivity treatments that are unique to MIH (36). Vital pulp therapy is validated by Al-Batayneh et al (32). to alleviate symptoms in impacted molars. To reduce perioperative hypersensitivity during dental treatments, Vicioni-Marques et al. (49) suggest preventive analgesia, which adds to MIH management options.

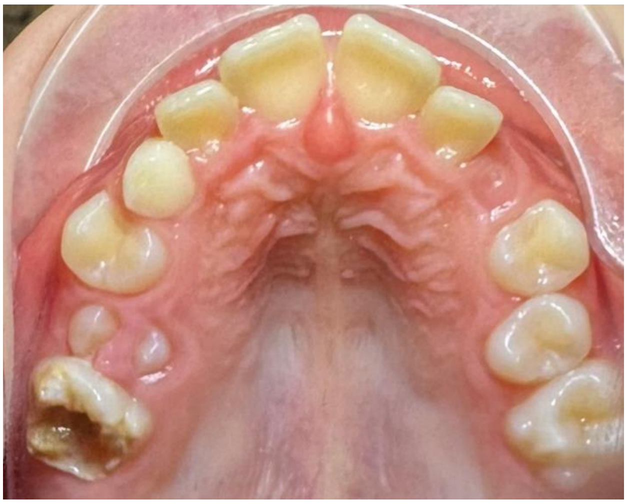

Fig. 2. *Examples of clinical manifestation of MIH.*

Dental sealants, fluoride paint application, and casein product use

Casein-based and Fluoride Varnish (FV) products are essential for preventing and treating molar incisor hypomineralization (MIH). By encouraging enamel remineralization, FVs lower the incidence of cavities and hypersensitivity. A calcium-phosphate-rich environment is maintained by casein phosphopeptide–amorphous calcium phosphate (CPP-ACP), which prevents demineralization and promotes remineralization (34). In a comparison of varnishes with 5% fluoride, fluoride with tricalcium phosphate, and CPP-ACP, Duraphat® proved to be the most successful for mild lesions, according to Biondi et al. (34). According to Restrepo et al. (46), applying FV decreased risk of caries and hypersensitivity. At the same time, it did not affect MIH lesions. For resin infiltration, Nogueira et al. (43) preferred it to FV. Sezer et al. (21) proposed a better remineralization capacity of CPP-ACFP.

According to Olgen et al. (44), all treatments enhanced remineralization; pastes, particularly, showed promise for long-term preservation, especially in yellow-brown lesions. These results highlight the importance of casein- and fluoride-based therapies in treating MIH and reducing its related consequences.

Restorations using composites and resin, direct and indirect

FVs and casein-based products are frequently used to treat molar incisor hypomineralization (MIH) to encourage remineralization and lessen hypersensitivity . Research by Biondi et al. (34) and Restrepo et al. (46) assessed how well different varnishes managed MIH lesions and increased mineral density. When comparing several treatment techniques, Nogueira et al. (43) preferred resin infiltration for better results. Sezer et al. (21) examined the possibility of remineralization with casein phosphopeptide-amorphous calcium fluoride phosphate (CPP-ACFP), indicating that it could be helpful under specific circumstances. Olgen et al. (44) noted encouraging outcomes with pastes that included phosphate and calcium ions, highlighting their function in long-term preservation. These studies emphasize the value of casein- and fluoride-based therapies in treating problems from MIH and protecting tooth structure.

Laser therapy at low levels

Sixty-six children with molar-incisor hypomineralization (MIH) participated in a randomized clinical trial by Muniz et al. (41) to evaluate the effects of fluoride varnish (FV), low-level laser therapy (LLLT) or both (L + FV). Comparable to the delayed impact of FV, LLLT provided instant alleviation, suggesting that it may help treat MIH-related sensitivity.

Treatment with fluorinated silver diamine

The treatment of MIH in children ages 6 to 13 was examined by Ballikaya et al. (33). They contrasted silver diammine fluorid by itself, SDF followed by silver modified atraumatic restorative technique (SMART) sealants in a split-mouth design. Both procedures successfully repaired the molars afflicted by MIH, while the SMART sealants showed some discoloration. The management of MIH in children appears to benefit from these minimally invasive approaches.

Mineralization through biomimicry

Treatment for MIH has traditionally focused on using sealants, fluoride, and restorations to manage clinical symptoms. However, developments in biomimetic mineralization present a chance to radically correct structural flaws in teeth impacted by MIH (38). This strategy seeks to repair dentin and enamel by imitating the mineralization mechanisms found in nature, potentially transforming MIH care beyond symptom management (39). Using materials and methods to direct the creation of enamel-like tissue, biomimetic mineralization aims to mimic the complex dynamics of enamel formation (45). Promising directions in this approach include customized treatment plans, tissue engineering methods, and enamel matrix proteins (47). Notwithstanding its potential, issues including the best biomaterials, long-term results, and accessibility still exist (1). Effective translation of these concepts into dental treatments requiring cooperation between researchers, material scientists, and practitioners is essential (48).

Limitations

Research on MIH treatment is limited by short trial durations, making evaluating long-term efficacy challenging. Comparing study results is made more difficult by disparate treatment approaches in research. Generalizability is limited by small sample sizes. Data interpretation is difficult when there are no standard parameters for the assessment. Furthermore, comprehension of treatment durability in many trials is hampered by inadequate long-term follow-up. Improving patient care and expanding our understanding of MIH treatments need to address these constraints.

CONCLUSIONS

Instead of using a one-size-fits-all strategy, MIH treatment should be customized based on the severity of each case, the patient's age, and other factors. Even with these developments, gaps in the scientific literature call for more investigation, especially in therapy stability and long-term efficacy. Discoveries should be incorporated into evidence-based clinical recommendations, emphasizing active patient and parental involvement for best results. MIH care necessitates an evidence-based, individualized approach, highlighting the need for continuous research to improve clinical recommendations.

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Conflicts of interest
The authors declare no conflict of interest.

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Letter to the Editor

THE 118 MEDICAL EMERGENCY CARE DURING COVID-19: NEW STRATEGIES FOR FUTURE PANDEMICS

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KEYWORDS: *118 SET Taranto ASL, Emergency Medicine, COVID-19, SARS-CoV-2, single nucleotide polymorphisms-SNPs, Long Covid*

INTRODUCTION

In Italy, acute life-threatening situations are handled by the 118 Emergency Service and pre-hospital medical facilities that rely upon centralized call reception, medical dispatching, and on-site emergency medical care before hospitalization care. The 118 services ensure that medical and paramedical units are often the first on the spot. At the same time, the professional skills of the dispatching physician are essential to ascertain the patient's needs to preserve life and vital functions while ensuring the appropriate emergency healthcare in the nearby hospital facilities.

The main reasons for suboptimal emergency care in life-threatening situations like the ones presented by the COVID-19 pandemic were a notch issue, with medical facilities being extremely reduced in some country areas, fewer volunteers and physicians, hospital reorganization, very tight funding, and the low skills of "first-on -the-scene" emergency workers. This raised the question of equal healthcare opportunity for everyone and the need for more qualified medical education, especially regarding emergency healthcare professionals, such as assistants, paramedicals, and drivers. COVID-19 urged an improvement in the system's efficiency with a plan for achieving objectives together with implementing a network of emergency services with varying degrees of emergency healthcare management.

In our experience, following the COVID-19 pandemic, the level of emergency healthcare opened up the necessity to operate with high-hand emergency equipment run by highly qualified emergency personnel also in considering the issues related to post-pandemic related problems, such could be the "long COVID" syndrome. This paper presented the strategic importance of the 118 nationwide during the recent pandemic, stressing the efforts and the countermeasures adopted by the 118 SET of Taranto City.

This letter wants to highlight that the territorial emergency health care provided by 118 National Units remains a key strategic player in emergency medicine in the field; however, there are still limitations due to the poor understanding of these values either from local authorities or central Government and poor ties between academics and 118 System in

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participating in data sharing. Therefore, it is necessary to promote dynamics to improve this practice both qualitatively and quantitatively to effectively be ready in any circumstances in the future (1).

The COVID-19 pandemic was one of the most significant challenges to the global healthcare systems, resulting in the sudden appearance of a large number of patients affected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and unveiled the grade of unpreparedness of the Italian health system. The healthcare providers not only needed to help numerous patients with a highly contagious disease, but they were also facing a shortage of care for patients with chronic diseases, emergency conditions, or other health conditions and diseases, and trying not to expose them to SARS-CoV-2 (2, 3).

In the early months of the coronavirus disease 2019 (COVID-19) pandemic, uncertainty surrounding the clinical course of patients and, importantly, the transmissibility of the pathogen presented significant operational challenges for paramedic services. Initial reports suggested that more than 40% of hospitalized patients require supplemental oxygen. As many as 15% may require mechanical ventilation (1-3), procedures for which there is an established risk of disease transmission to healthcare providers through infectious aerosols (4).

Indeed, the initial months of the pandemic saw shocking rates of infections and deaths among healthcare workers, doctors, nurses, and paramedics caring for patients with COVID-19 (5, 6). This risk of infection was practically even more pronounced owing to the extreme difficulties of providing care in a prehospital setting, where environments were difficult to control, patients were undifferentiated, Personal Protective Equipment (PPE), resources were limited, and information was scarce can make wearing. During the lockdown period, recorded home infection events increased significantly (5, 6). However, little scientific evidence has been produced on the impact of the lockdown on out-of-hospital deaths related to COVID-19. Significant changes performed in the Emergency Medical Services (EMS) during the pandemic did not cause a substantial increase in mortality from major trauma in our large study population (5-7).

Context

The Taranto Provincial Operations Center "118" Territorial Emergency System (SET 118) is the publicly funded provider of land ambulance and medic/paramedic services to the municipalities of Taranto city and its provinces in the Apulia region, collectively encompassing a mixed suburban and rural geography of 2467,35 km^2, with a population of 600.000. The service employs more than 700 Primary and Advanced Care Paramedics and Medics who staff 65 ambulances and eight rapid response units during peak hours (Fig. 1) (8).

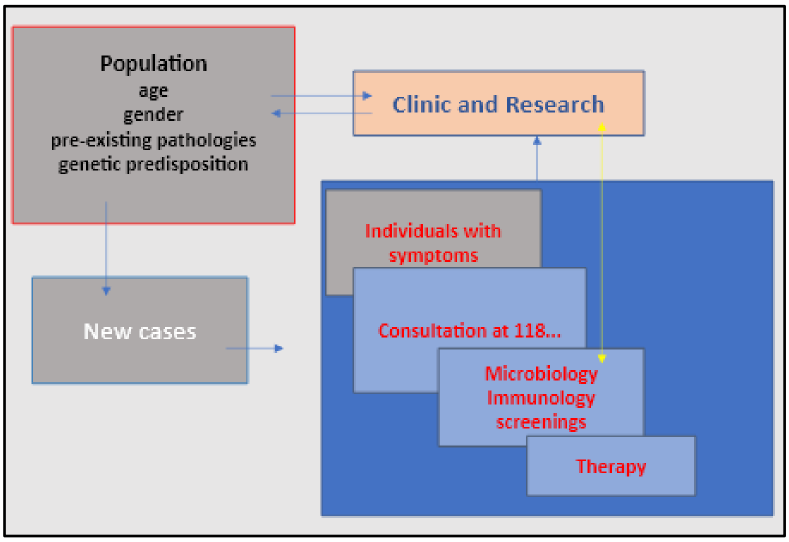

Fig. 1. *The gate model proposed by Taranto's SET 118 assigns a unique role of protection of the territory both to the 118 doctors and to the GPs, according to the Anglo-Saxon logic of entrusting the specialist reception unit of the hospital: the GPs and the doctors of the 118 have been proposed as the diagnostic vanguard to face and solve the first problems acting as filters before being directed to specialist cares. This way, a new management procedure was emphasized to coordinate territory and hospital care to implement a systematic therapeutic plan.*

The Province of Taranto is geographically divided into 6 health districts, each with a centralized headquarters facility from which crews start their shift in a 'hub-and-spoke' and are then required by 118 calls to move to the territories or required spots. Ambulances are usually crewed by two paramedics, one in primary care (PCP) and one in advanced care (ACP); rapid response units are crewed by a single PCP. On average, 118 can respond to approximately 130,000 emergency calls annually (9).

In April 2020, 118 were operating within a declared state of emergency in Italy, while the SET 118 Taranto mobilized a joint union and management Task Force under the incident management system framework to develop the local rescue. Our task force included representatives with medic and paramedic education expertise, occupational health nurses and safety professionals, and an immunology team liaising with the Emergency Operations Center and the Taranto Central Health Department Service's response to the crisis (8). SET 118 Taranto has persisted as one of several 'hot spots' in the province for taking care of community transmission of COVID-19, owing, in part, to essential emergency staff and territorial units (8, 10).

Interventions, program, and objectives

In April 2020, operating within a declared state of emergency in the Apulia region, local hospital SG Moscati and 118 SET mobilized a joint union and management Task Force by setting up a filter unit under the 118-management system framework to develop a first emergency rescue before the hospital acceptance. The task force included paramedics, nurses and doctors, medical immunology researchers, and service operations, serving as the liaison with the Emergency Operations Center of the Province and Region (10).

Over 1500 patients were admitted to our COVID-19 Emergency Unit SET 118 of the SG Moscati Hospital in Taranto City from March 2020 to November 2020. The majority were males (61%), 17 deceased (5%), and had an average age of 72. The 118 pre-hospital health systems operating in the province of Taranto received over 10.000 calls from patients who reported symptoms attributable to possible respiratory conditions or who were accused of having contact with people with suspected or acclaimed SARS-CoV-2 infection. 40.96% of calls were for female patients, while 59.04% were for male patients (10, 11).

Reported symptoms were fever, cough, general malaise, breathing difficulties, headache, cold, sore throat, conjunctivitis, taste and/or smell alterations, and gastrointestinal symptoms; a copious percentage of them reported to have had contact with SARS CoV-2 patients without manifesting infection symptoms. In addition, patients reporting symptoms were divided into different age groups: 0-9 years, 10-19 years, 20-34 years, 35 -59 years, 60-69 years, 70-79 years, 80-89 years, and≥ 90 years.

The dynamic development of the hospital preparedness and the SET 118 response was essential to ensure the proper healthcare efficacy due to COVID-19, to reduce the spread of the infection, and prevent hospitals from becoming overwhelmed due to significant numbers of critically ill patients infected with COVID-19 (10-12). A key consideration was the 118's resilience and ability to adapt and manage beyond what was usually possible to provide a pre-hospital clinical treatment, as COVID-19 resulted in a rapidly rising demand. Therefore, the Taranto SET 118 was required to take proactive measures to manage emergency care demands, identify the gaps in territorial critical care, and identify maximal case admission capacities (10, 11).

Our model used data that included City Performance Indicators such as daily emergency call types, clinical priority rank, ambulance types (Dual/ALS/BLS), and Emergency Medical Technicians (EMT)/paramedic or medic crew data often collected during previous non-pandemic periods. Second, we addressed the possible limitations encountered from other COVID studies that may include recognition and statements of early signs and symptoms parameters (fever, cough, breathing difficulties, loss of taste and loss of smell, extreme fatigue, etc.) (10-12).

The overall program goal for the SET 118 Taranto was to prevent community COVID-19 infections and hospital congestion while continuing to provide high-quality resuscitative care to patients. In support of that goal, we identified three specific program objectives: 1) limit medic and paramedic exposure to the virus within the service to the greatest extent reasonably possible; 2) ensure the hospital’s services and safety through frequent exposure to high-risk patient types and procedures; 3) provide high-quality resuscitative care to critically ill patients, including the performance of aerosolizing procedures where they were typically indicated avoiding to overload Hospital’s care units (10).

Based on that intent, we proceeded to fix the underlying logistic and medical intervention problems based on some previous studies and our experiences before the COVID-19 pandemic. Therefore, the first move was to determine the relative contribution of medical emergencies and other factors in predicting ambulance travel or response times using a coordinated system of information changing between fields and a central operative quarter (COQ). It was settled on a specific intervention time frame following the relationship between the number of calls or counts and duration (the time that has elapsed between calls) with the collected data and symptoms described by the patients. Between each call and intervention, ambulances and first responders were dispatched to each event, and the critical timing of each intervention was recorded.

Innovative operational concept

Early in the pandemic, the regulatory bodies that govern SET 118 practices in Apulia released a regulation that recommended curtailing the ordinary mansions in all but extraordinary circumstances. This included limiting the use of nebulized medications, continuous positive airway pressure, bag-mask ventilation, and high-flow oxygen administration (10-12).

The 118 SET's emergency response system to the COVID-19 pandemic consisted of multiple interconnected components, such as the creation of a pre-hospital special COVID-19 unit functioning as in-bed clinical treatment, the creation of an emergency management plan that included the infection prevention and control program, the creation of a special research unit to collect data, numbers, and scientific information to support clinical case management, communication coordination, laboratory and diagnostic services (10-12).

We, therefore, conducted a systematic narrative review of the emerging literature while implementing a broad medical research activity that allowed us to get out with new diagnostic and treatment methodologies. The final results allowed us, in a brief time, to reach a comprehensive picture regarding the SARS-CoV-2 pleiotropism and describe recommendations for COVID-19 management, cardiopulmonary resuscitation, and preventive measurements in patients confirmed or suspected to have COVID-19 and the general population. The research and the new descriptive approach allowed us to distill a straightforward protocol that informed and prepared our operations in the field and COVID-19 UNIT.

In considering these recommendations, we were able to structure the team to achieve most of them, with the notable exception of using video laryngoscopes for intubation, which is not standard in our daily service and procedure.

Finally, we drew on the best practices related to human factors used by high-performing emergency medical services systems in Europe, such as the use of pre-procedure checklists and principles of crisis resource management.

Our task force developed the operational concept and procedures for the team through consensus building based on the emerging literature and best practice recommendations described above. This required regular in-person meetings, frequent engagement with ambulance staff and front-line paramedics and supervisors from other departments (ICU, Pneumology, and Infectious Disease Department) within the paramedic service, and simulating proposed procedures to identify potential failure points.

The SET 118 COVID-19 filter unit

We designed the COVID-19 Special Unit equipped with a negative pressure system to deploy the immense flow of patients into SG Moscati hospital, opened 24h per day. In a departure from normal operations, the unit’s crews were utilized outside regular dispatching procedures. They were autonomous in selecting patients to attend based on physical and diagnostics parameters to identify high-acuity patients who were likely to require first aerosolizing and medical rescue procedures. The crews received text message alerts from our ambulance or in-field team for calls involving 'clear, immediate threat to life' criteria, including dry coughing, fever, cardiac arrest, altered level of consciousness, severe respiratory distress, and existing co-morbidities, among others. Doctors and nurses in the acceptance could additionally request a COVID-19 Special Unit crew to attend at the scene.

The COVID-19 Special Unit was intended to be a promptly arriving 'first-in' resource. The medical crew assessed the risk assessment with the present paramedics while patients completed the required test and diagnostic procedure. Of course, it was adapted a special '*COVID Zone*' divided into two designated wings, “A area” in which only our special personnel were present during patient care, and a 'B area or Warm Zone' where critical care patients in airborne personal protective equipment (PPE) would stay requiring high O_2 flux masks.

Once any aerosolizing procedures had been completed, staff at the scene would assist with mask extrication, and the patient would be transported to the main hospital’s departments. At the COVID-19 SET 118 Unit, the medical officer was responsible for liaising with the receiving facility staff to settle the transfer procedure and a detailed care plan before the patient was removed from the structure (Fig. 2, 3).

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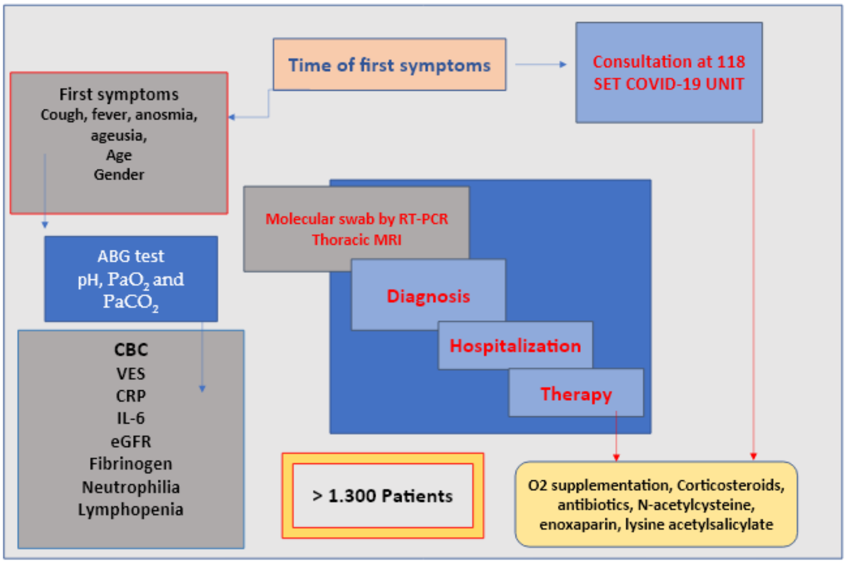

Fig. 2. *The algorithm allowed the 118 SET to reach tremendous results, proceeding with a multi-disciplinary approach by setting up a multi-centered therapy that combined O_2 support, antioxidants, antiretroviral, cortisone, and antibiotics to arrest systemic inflammation and prevent multi organs decay. This model was based on an epidemic that prevented action for the upcoming winter season.*

Prehospital emergency care of suspected COVID-19 patient with acute respiratory failure

Objective

Ensure the patient classified as a suspected or full-blown case with an initial clinical picture of acute respiratory failure and/or shock the appropriate and continued emergency therapeutic support during the phases of protected transport and temporary management pending the taking charge of the dedicated hospital units.

Methodology

At home and in a mobile station (ambulance) SET-118 → acute respiratory failure → therapeutic protocol

Oxygen therapy, as needed (SpO2> 90%):

- low flow (P/F > 300 mmHg):
  with nasal goggles: 2 – 4 L/min

- high flow (P/F < 300 mmHg):
  with face mask with reservoir: 15 L/min

Non Invasive Mechanical Ventilation (SpO2< 90% or P/F < 200 mmHg + severe dyspnoea, use of accessory respiratory muscles - sternocleidomastoid, scalenes, paradoxical breathing-, RR > 35 breaths/min, pH < 7.35, pH> 7.2, Kelly 1-2)
→ CPAP: 5 – 10 cm H2O, with FiO2 of 60 – 90%.

In more severe cases

In the presence of severe hypercapnia, altered mental status, hemodynamic instability, invasive mechanical ventilation IMV → ETI is indicated.
If the clinical picture compatible with bilateral interstitial pneumonia:
dexamethasone: 6 mg iv (associated with gastroprotection with pantoprazole 40 mg iv)
acetylcysteine fl300 mg iv: 2 flev in 250 ml of saline
enoxaparinafl: 1 fl4000 IU sc (in the absence of specific contraindications)
Intravenous drip with 5% glucose solution→ for nutritional purposes, in case of prolonged hospitalization.

Where an emergency vehicle with a non-medicalized but nursed crew intervenes on an unstable COVID-19 patient, the 118 Operations Center can guarantee remotely, through the CO118 doctor or even through the SET doctor specifically dedicated in service at the CO118 to carry out operations of "medical control online", real-time medical support for the administration of emergency therapy.

Fig. 3. *The admission to the pre-hospital unit was created to avoid overloading the hospital in the Taranto areas. The admission phases were based on each patient's clinical condition at acceptance into the 118 COVID-19 Unit. Based on symptoms and ABG parameters, the therapies could be delivered home, organized, and performed at the site.*

Measures, analysis, and research, what we have achieved

There was a lack of evidence on how the measures were obtained, modified, and adapted according to the area and population as the pandemic progressed. However, some researchers and labs have already revealed changes in use with recoveries and relapses during the advanced stages of the pandemic, such as the long-covid cases. Therefore, our evaluation, screening, and research program consisted of a manual review of data of all electronic patient care records (ePCRs), compelling lab results conducted with peer review research of published work on virus, microbiology, and immunology regarding similar virus infection. We were specifically interested in the proportion of calls involving high-risk COVID-19 to predict, prevent, and treat the COVID-19 infection (12, 13).

To provide a contextualized basis of comparison, we also manually reviewed the ePCRs for all admissions in which COVID-19 patients were performed in the first and second waves (February-May 2020 and September-November 2020) of the pandemic leading up to the drafting of articles for international publication. Following the conclusion of several manuscripts, we proceeded to explore either a scientific hypothesis related to SARS-CoV-2 unique pleiotropism or to create a new RT-PCR diagnostic method as a result of working on teams with different departments, laboratories, and Universities (national and international)such as Aldo Moro University of Bari (Italy), Phan Chau Trinh University of Medicine (Vietnam) and Lincoln University (Oakland California-USA).

Thanks to this approach, we soon collected critical information that gave us a clear picture of the situation. The SARS-CoV-2 slowly revealed its pathogenesis traits, helping us to assess faster diagnosis and perform more efficacious therapy. The results confirmed distinctive patterns of how the virus infected and its ability to avoid the host's immune surveillance and responses (12, 13).

Our data regarding the gender-based differences in COVID-19 suggested that male patients were at higher risk of developing severe disease with an increased rate of mortality, and intervening time was crucial, 36 hours maximum from the first signs. The results from the retrospective cohort studies from March to November 2020, evaluating the mortality rate in over 1300 acute care patients with confirmed SARS-CoV-2 infection, reported a higher mortality rate in male patients (12.5%) compared to female patients (9.6%) (8, 10).

We were among the first to discuss the "happy hypoxia" phenomenon, coined by J. Couzin-Frankel (8, 14). The "happy hypoxia" was an event that many patients experienced, characterized by a sudden decay rescued by high flux oxygen support performed in the ICU department. The etiopathogenesis was then correlated to severe endothelitis, considered a major causative factor affecting the microcirculatory mechanism, followed by a silent and rapid necrosis process linked to a generalized rise of uncontrolled inflammatory processes that lead to microvascular thrombosis, coma, and then death (8, 14, 15).

Due to frequent events of sudden deterioration of many patients, we started implementing the use of arterial blood gas analysis (ABG), which helped to clarify a few specific features of the infection, allowing us to adopt more efficacious therapy and treatments. The outcomes from the ABG were atypical, indicating an acute hypercapnic respiratory state accompanied by a hypoxemic condition with a compensatory alkalosis, which was suggestive of a progressive pulmonary micro embolism, specular of an internal ongoing hypercoagulability with endothelial activation as a consequence of an uncontrolled increase in proinflammatory cytokines, the notorious "cytokine storm" (16, 17).

The hypoxaemic state was described as an increase in minute ventilation that drives to uncontrolled hypocapnia; due to the CO2's fast diffusion into the tissues, the CO_2 moves about 20 times faster than O_2. By this point, we were able to understand the pathoanatomical and pathophysiological basis of COVID-19 respiratory failure, characterized by the presence of progressive and diffuse multi-organ tissue and alveolar damages with interstitial thickening, deep venous thromboembolisms, and gas exchange impairments. A scenario often accompanied by atelectasis and lung consolidations was seen in CT-scan images with typical ground glass opacities (8, 10).

Eventually, the combination of ABG analysis (the oxygen and carbon dioxide partial saturation level-PaO_2 and $PaCO_2$) and the CT scan proved to be a better tool for diagnosing COVID-19 than swab RT-PCR alone (8, 10).

The COVID-19 patients' cell blood count (CBC) performed immediately after the ABG tests indicated the presence of an infectious inflammatory condition with the involvement of the lungs, heart, and kidneys. Most patients revealed a high white cell count (WBC > 10.000 cells/mcL), with marked neutrophilia and lymphopenia. Laboratory outcomes confirmed low eGFR and 25OH-vitamin D levels, increased troponin, IL-6, D-Dimer, and erythrocyte sedimentation rate (ESR) levels, and an augmented fibrinogen level. At that time, we could point out a secondary phase of the infection triggered by aggressive bacteria, which was confirmed by other teams worldwide. The rapidity of multiorgan involvement with the contextual septic course was, in fact, related to the presence of different pathogens identified in BALF and blood culture, such as *Klebsiella spp*, *Candida albicans*, *Aspergillus*, *Pseudomonas spp*, that showed to be a prerogative trait of the conclusive phase of SARS-CoV-2 infection (8, 10).

The mechanism of Sars-CoV-2 infection was confirmed to affect any type of cells (epithelial, neurons, myocytes, etc.) via the angiotensin-converting enzyme-2 (ACE2), followed by the cleavage of S by the transmembrane protease serine 2 (TMPRSS2). which explains the diversity of symptoms that characterize COVID-19 disease (8, 10, 14, 15) (16).

Our investigation allowed us to explain very unusual phenomena, consisting of the aberrant increase of erythroid progenitors, characterized by an abnormal decrease in platelet circulation in critical hospitalized cases or after receiving the vaccine shot (18). Those observations, together with hypoxia, hypocapnia, alkalosis, iron anemia, and coagulopathies, were seen as highly correlated with an alarming degree of death risk (16). Intriguingly, we were able to highlight the increased mean platelet volume (MPV) and platelet hyperactivity that we often encountered in COVID-19 patients, with a decreased level in the overall platelet count since the progenitors of the erythroid and myeloid lineage appear to be the only cell types expressing both ACE2 and TMPRSS2 among the cells present in bone marrow (9, 16-20). Events that eventually proved the contamination of erythroid lineage by the virus during the differentiation phase and the decreased number of platelets due to autoimmune attack by T cells, neutrophils, and NK cells (9, 17-20) (Fig. 4).

The typical COVID-19 patient

anosmia, ageusia, light fever, light headache and dry cough, deep fatigue;
marked alkalotic, hypoxic, hypocapnia, the ABG profile with hyperventilation at the time of admission;
the laboratory and microbiology results showed lymphopenia, neutrophilia;
fibrinogen, ESR, CRP, vitamin D and eGFR were markedly anomalous;
markedly high IL-6 levels;
thrombocytopenia, anemia;
BALF showed the presence of few opportunistic pathogens *Klebsiella spp, Candida albicans, Aspergillus, Pseudomonas spp*
Total number of CD4+ and CD8+ T cells showed a drastic decrease in COVID-19 patients with levels lower than the normal range delimited by 400/μL and 800/μL, respectively, and were negatively correlated with blood inflammatory responses; low level of B lymphocytes, low level of T-reg CD4+CD25+high and high level of T killer cells, high level of CD8+CD57+ suppressor, high level of CD8+ CD38+DR+, and monocytes were seen in COVID-19 patients
Patient with mild to severe COVID-19 infection revealed the carry a precise genetic make-up of SNPs related to those genes regulating the immune responses

Fig. 4. *The overall picture of affected patients was a result that we reached gradually based on the clinical, laboratory, and investigative approaches.*

The panel of considered cytokines also revealed an association with hospitalization time, age, and sex. The cytokine signatures associated with patients were partially. They included molecules that have been implicated in COVID-19 pathogenesis, such as IL-6, eGFR, vitamin D3, and fibrinogen, as well as molecules that are more generally associated with inflammation/infection, such as ESR, D-dimer, PCR, and iron (21). Overall, the cell-mediated immune response was significantly up-regulated in COVID-19 cases compared to other patients. In this case, we assumed the necessary existence of lung–kidney–heart cross-talk, which eventually explains simultaneously the whole complexity of COVID-19 disease and its progression mechanism together with the uncontrolled autoimmune responses under the guidance of the IL-6 that leads to the well-known “cytokine storm” (1-5, 13-16).

The data obtained from our analysis aligned with the outcomes of observational studies that confirmed that a decreased vitamin D level, in a concentration lower than 20 ng/mL, was a distinctive trait in COVID-19 patients and correlated to a negative prognosis (19).

Our primary clinical findings showed an evident reduction of platelets and erythrocytes, an event related to the deficit seen in immune system response towards own affected cells infected by the SARS-CoV-2. Initial measures were generally centered on containing the spread of auto-immune reactions and uncontrolled inflammatory flares, providing a therapy centered on both anti-inflammatory, anti-oxidants and two types of antibiotics to arrest a secondary kind of bacteria infection often associated with COVID-19 to meet the soaring need for lost immune modulation as a consequent reduction in lacking immune regulatory cells and cytokines (3, 4, 7, 10, 12). Results were highly suggestive of profound compromised immune system during SARS-CoV-2 infection, lymphopenia (64%), low level of B-lymphocytes (60%), low level of CD4 $CD25^{high}$ regulatory T cells (37.8%) and high level of T killer cells (73.3%), high level of CD8+CD57+ suppressor (64.44%), high level of CD8+CD38+DR+ (80%), and monocytes (28.9%) were evident features seen in COVID-19 patients (16).

As lung capacity starts decaying due to microvascular homeostasis deficiency, cardiovascular functions also worsen, affecting the reuptake of filtered 25-hydroxyvitamin D in kidney proximal tubules (9, 18, 21). The vitamin D has endocrine, paracrine, and autocrine functions and is capable of inhibiting the RAS due to its involvement in preventing angiotensin II (Ang-II) accumulation via the inhibition of renin release, a widespread event in COVID-19 patients (21, 22). The rise of Ang-II in cholesterol plaque accumulation along arteries, veins, and visceral glomerular epithelial cells (podocytes) is a well-known phenomenon that induces cholesterol metabolism dysfunction, leading to kidney and cardiovascular injuries (21). In this scenario, we considered the augmented toxic effects of the spike protein known to promote Ang II accumulation, which explains cardiac hypertrophy and cardiac failure; A): Ang-II is produced within the myocardium; B): Ang-II is activated within the failing hypertrophic heart; and (C): the pharmacological inhibition of the RAS and Ang-II in animal models and patients with a hypertrophic heart experiencing a myocardial failure showed to be highly effective (23-26).

One of the most confusing traits of the COVID-19 infection was the significant disparity of affected individuals. The exposure to the virus could not explain the variety of responses to the virus and the considerable differences between those who showed the disease and those who, conversely, did not, despite their direct contact with the infected ones. While a person's pre-existing condition and their immune defenses were soon confirmed to play a key role in the disease progression, there was the need to explain the hosts' genetic makeup towards COVID-19 susceptibility and risk (20, 21). The genetic test results performed on patients eventually confirmed this position; the host’s genetic makeup was shown to influence the degree of predisposition and outcomes of COVID-19. One of the best achievements in this direction was to investigate and highlight the presence of single nucleotide polymorphisms (SNPs) of those genes involved in the immune regulatory mechanism. The disease grade of severity was soon observed concerning the presence of specific SNPs (9). The overall outcomes showed the following: ACE-1 (I/D higher prevalence in COVID-19 group), Serpina3 (G/T higher prevalence in COVID-19 group), CRP (G/G higher prevalence in the healthy group), IL6 rs1800795 (G/G-G/C higher prevalence in COVID-19 group), and IL10 (G/A higher prevalence in healthy group; A/A higher prevalence in COVID-19 group) and IL1RN (C/T-T/T higher prevalence in COVID-19 group; C/C higher prevalence in the healthy group), IL6R (A/A lower prevalence in COVID-19 group), VDR (Fok1 TC higher prevalence in COVID-19 group, and T/T higher prevalence in the healthy group; Taq1 A/G higher prevalence in COVID-19 group, G/G higher prevalence in healthy group), IFNγ (A/A lower prevalence in COVID-19 group, A/T higher prevalence in healthy group), and TNFα (G/G higher prevalence in COVID-19 group) (Fig. 3) (9, 20).

Final consideration and the problem of long COVID

The COVID pandemic has taught the importance of multidisciplinary collaboration for effective diagnosis, treatment, and prevention (26-28). This has also required cooperation between primary emergency and secondary care, which involved collaboration between departments and disciplines such as emergency medicine, immunology, and microbiology (29-31). Among those patients affected by COVID-19, many showed comorbidities at admission, but many revealed the long-term effects of the infection, later named Long COVID syndrome; these patients showed a very particular clinical picture and were often seriously ill.

The management of patients with comorbidities and long-term COVID-19 requires a multi-disciplinary team-based approach and treatment to achieve a complete recovery (32). Clinical presentations have been recently described in patients some months after being affected by COVID-19 (32, 33). Similar descriptions can be found in the literature and vary from foggy thoughts, anemia, neurasthenia, vegetative neuritis, post-viral fatigue syndrome, sleeping disorders, raphe nucleus encephalopathy, chronic lung condition (lung interstitial fibrotic scars), and chronic mononucleosis syndrome among others (32, 33). This virus (RNA-virus) and the vaccines (mRNA), which are analogous, may start with uncontrolled immune responses lead to extreme "central" decay a picture which also includes a reduction in B lymphocytes, increasing pro-inflammatory cytokines (IL-6, IL8, TNFa), rising of glial macrophages (M1) (responsible neural inflammation) and activation of self-reactive T-cells (Cytotoxic T cells and Th1) (32, 33).

These findings confirm that post-COVID conditions will indeed represent another challenge for healthcare professionals since these signs should also be referred to as pre-existent comorbidities deeply linked with a higher related burden (32). The fact that this variegate symptomatic picture would indicate a common Long-COVID condition eventually led to considering the specific role of SARS-CoV-2 variants. We therefore suggest that pathogenic SARS-CoV-2 spike protein and the cell-to-cell mechanisms associated with the development of Long-COVID syndrome could be similar but not the same among SARS-CoV-2 different variants (32).

General data and outcomes would support this assumption; individuals infected with the first variants exhibited a more significant number of post-COVID symptoms, particularly respiratory symptoms such as dyspnea, feverish, and fatigue, than those patients infected with the following variants. Many exhibited lung fibrotic tissues validated by thoracic CT scan even after 12 months from the COVID-19 infection (34, 35).

However, the only exposure to the virus could not sustain the great variety of each individual's responses to the virus and the vast diversity of signs and symptoms (20). Accordingly, it is expected that the development of infection and post-infection symptoms will be higher not only in the presence of historical variants and pre-existing conditions but also with mRNA vaccines. All are in a predisposing genetic environment with a well-defined genetic makeup (20, 21, 33, 34, 36).

They were implementing innovative, although complex, strategies, research, and clinical procedures to address and solve the abovementioned problems, which were not simple. However, despite the difficulties, the 118 SET proved that this approach effectively reduced mortality and generated new effective and deliberative discussions on the COVID-19 pandemic, stimulating new perspectives and concrete actions about plans, priorities, and strategies (33, 34, 36, 37).

The proposed method helped to keep everyone engaged and raise awareness and questions, allowing the set-up of concrete targets to be reached step by step for a wide variety of healthcare issues. Our health services' complex challenges were enormous and impossible without a multidisciplinary interchange and debate. The current approach was of great help in standardizing health procedures and improved network governance in Taranto province, offering a proven method to strengthen the impact of health services on population health, which in the post-COVID era is more necessary than ever.

Therefore, with this paper, we propose a new procedure that might become a “Know-how” tool to reach the formation of well-organized interdisciplinary teams of healthcare professionals. This has proved to be an effective way of proceeding during the pandemic, and we profoundly hope it will be used to help health professionals solve upcoming new planetary challenges.

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