Bacterial Resistance in Hospital-Acquired Infections Acquired in the Intensive Care Unit: A Systematic Review

Walter Martinez Loaiza[1], Anny Katheryne Rivera Ruiz[1], Cristian Camilo Ospina Patiño[1], Mónica Chavez Vivas[2,3,*]

ABSTRACT

Purpose: In this review we present the status of the prevalence of bacteria resistant to antibiotics and the main antibiotic resistance genes that are reported in infections acquired in intensive care units (ICU) around the world.

Methods: A systematic review based on the PRISMA guide was carried out, from the Science Direct, Redalyc, Scopus, Hinari, Scielo, Dialnet, PLOS, ProQuest, Taylor, Lilacs and PubMed/Medline databases. Inclusion criteria of this review were original research study published in a scientific journal in a 10-year time span from 1 January 2017 and 30 April 2022.

Results: A total of 1686 studies were identified, but only 114 studies were considered eligible for inclusion. *Klebsiella pneumoniae* and *Escherichia coli* resistant to carbapenems and producers of extended-spectrum β-lactamases (ESBL) are the most frequently isolated pathogens in ICUs in Asia, Africa and Latin America. The *bla*OXA and *bla*CTX were antibiotic resistance genes (ARG) most commonly reported in different geographic regions (in 30 and 28 studies, respectively). Moreover, multidrug-resistant (MDR) strains were reported in higher frequency in hospital-acquired infections. Reports of MDR strains vary between continents, with the majority of publications being in Asia and between countries, with Egypt and Iran being highlighted. There is a predominance of few bacterial clones with MDR phenotype, for example, clonal complex 5 Methicillin-Resistant *Staphylococcus aureus* (CC5-MRSA) circulates frequently in hospitals in the United States, clone ST23-*K. pneumoniae* is reported in India and Iran, and clone ST260 carbapenemase-producing *P. aeruginosa* in the United States and Estonia.

Conclusion: Our systematic review reveals that ESBL- and carbapenemase-producing *K. pneumoniae* and *E. coli* are the most problematic bacteria that are reported, mainly in tertiary hospitals in Asia, Africa, and Latin America. We have also found propagation of dominant clones with a high degree of MDR, becoming a problem due to its high capacity to cause morbidity, mortality and additional hospital costs.

KEYWORDS

drug resistance; antibiotic resistant bacteria; antibiotic resistance genes; intensive care units

AUTHOR AFFILIATIONS

[1] Medicine Program, Faculty of Health, Universidad Santiago de Cali, Colombia
[2] Medicine Program, Faculty of Health Sciences, Universidad Libre, Cali, Colombia
[3] Investigation Group GIMMEIN, Colombia
* Corresponding author: Campus Valle del Lili, Carrera 109 No. 22-00 – Valle del Lili A.A. 1040, Cali, Colombia; e-mail: monikchavez@gmail.com, monica.chavezv@unilibre.edu.co

Received: 29 June 2022
Accepted: 1 March 2023
Published online: 26 June 2023

Acta Medica (Hradec Králové) 2023; 66(1): 1–10
https://doi.org/10.14712/18059694.2023.8

INTRODUCTION

Antibiotic resistance is defined as the ability of the bacterium to avoid the action of the antibiotic, which can be done by modifying target proteins due to point mutations or by acquisition of resistance genes through mobile genetic elements (1–5). This resistance can be accelerated by the incorrect and indiscriminate use of these drugs, which leads to multiple resistances in different strains of bacteria, with the consequent increase in hospital-acquired infections (6–8), that can have great influence to the health of the world population.

In the last decade, the increase in antimicrobial resistance in ICUs has been reported, mainly due to the spread of these multidrug-resistant (MDR) bacteria (8–12). MDR is defined as resistance to more than one agent in three or more antimicrobial categories, extensively-drug resistant bacteria (XDR), is defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e., bacterial isolates remain susceptible to only one or two categories), and pan-drug resistant bacteria (PDR) is defined as non-susceptibility to all agents in all antimicrobial categories (9). The situation is complicated by the presence of so-called "High-Risk Clones (HiRCs)", which corresponds to few lineages of bacteria that have the ability to adapt and remain for long periods of time in the hospital environment. Some of these clones would be involved in the appearance of resistance mechanisms that affect new antimicrobials. The development and speed of spread of HiRCs would have been potentiated by the high use of all antibiotics during the COVID-19 pandemic, as proposed by several researchers (13–15).

The risk factor of development of infection caused by antibiotic-resistant bacteria is hospital stay, especially in ICU. Patients in these facilities usually receive intensive antibiotic therapy and a lot of hands-on care, and their special condition makes them vulnerable to acquiring bacteria with various types of resistance (15, 16).

The objective of this review was to find the status of prevalence of bacteria resistant to antibiotics caused an infection in ICU around the world. The second aim was to find what antibiotic resistance genes (ARG) are reported in the same infections acquired in ICU, in order to contribute to the strengthening of antibiotic resistance control policies.

METHODS

Systematic search of various electronic databases such Science Direct (Elsevier), Redalyc, Scopus, Hinari, Scielo, Dialnet, PLOS, ProQuest, Taylor, Lilacs and PubMed/Medline was conducted to retrieve relevant published articles. Online library repositories of different institutions were also searched. The process of retrieving and including data closely followed PRISMA guidelines (Preferred Reporting Items of Systematic Reviews and Meta-Analyses) as shown in Figure 1. Relevant MeSH terms and keywords were used

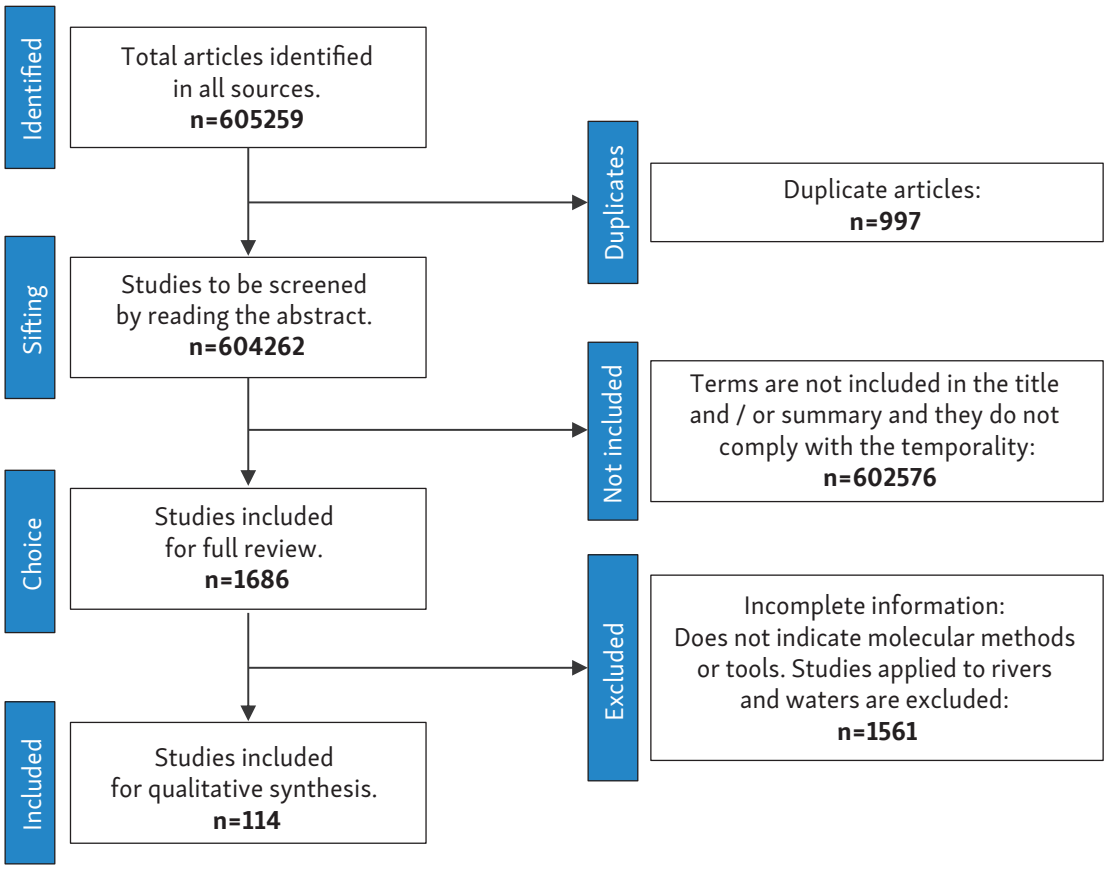

Fig. 1 Algorithm for the literature review.

to retrieve all relevant articles from the above-listed databases. The keywords and MeSH terms used were: "antibiotic resistance", "antimicrobial resistant strains", "Multidrug-resistant", "antibiotic resistant bacteria", "antibiotic resistance genes (ARG)", and "hospital-acquired infections". Studies published from 1 January 2017 and 30 April 2022 were included. We excluded review articles, systematic review, meta-analyses, editorials, policy statements, research exclusively in child populations, and those with data collection commencing prior to 2017. A full list of the data elements extracted from each study are reported in supplementary material.

RESULTS AND DISCUSSION

STUDY CHARACTERISTICS

Out of a total of 1686 unique records were screened, 114 studies met our inclusion criteria (Fig. 1). The maximum number of studies were found in Asia (n = 42), of which nine (7.9%) were conducted in China. From studies with specific diseases, the most common sample were urine (n = 92), blood (n = 86) and respiratory secretions (n = 76).

Most of the articles report bacteria with resistance to antibiotics based on conventional methods (as disk diffusion method, Double disc synergy test, dilution methods, Epsilometer test), especially in countries of Africa (2, 11, 17–34), Asia (35–60) and Latin America (1, 6, 61–66). Phenotypic detection of antibiotic resistance by Disk Diffusion Method was reported in 60.5% of the total studies, followed by the Vitek 2 system (18.4%). Most studies (79.6%) used the CLSI as the breakpoint reference guidelines (18.4%) (Table 1). The most commonly used molecular methods for the study of bacterial resistance corresponded to the conventional PCR technique (refers to the basic type of PCR reaction) (40.4%). A low number of reports (11.4%) were found that use last generation molecular methods (such as, Next Generation Sequencing, which is the large-scale DNA sequencing technology that allows the analysis of entire genomes or specific genes).

DISTRIBUTION OF ISOLATES

Figure 2 shows distribution of bacterial species in clinical samples. *K. pneumoniae* (n = 57) and *E. coli* (n = 51) were the most reported bacteria, especially in urine samples,

Tab. 1 The number of studies about bacterial identification method, phenotypic and molecular detection method in the present systematic review.

Characteristics	No of studies	References
Bacterial Identification method		
Morphology / Biochemical testing	30 (26.3%)	1–4, 6, 11, 13, 15, 17–21, 34–42, 60–62, 66, 67
API	7 (6.1%)	21, 27–30, 75, 76
VITEK®	17 (14.9%)	4, 14, 15, 25, 35, 66, 75, 77–86
MALDI-TOF	18 (15.8%)	5, 12, 14, 15, 22, 66, 67, 69, 71, 73, 74, 76, 79, 87–90
COMBO DISC, QUBIT® 2.0 FLUOROMETER	1 (0.9%)	91
Not mentioned	1 (0.9%)	92
Phenotypic detection method		
Disk Diffusion Method (Kirby Bauer disk diffusion method / Mueller Hinton agar)	69 (60.5%)	2–4, 6–9, 11, 12, 14, 16–37, 39–59, 61–74
Double disc synergy test	2 (1.8%)	26, 29
Dilution / test-broth microdilution / MicroScan autoSCAN-4 automated System	18 (15.8%)	12, 13, 70–74, 77, 80, 89, 93–100
E Test	3 (2.6%)	21, 38, 70
VITEK® 2	21 (18.4%)	14, 15, 25, 34, 75, 78, 79, 87, 89, 101–111
Neo-Rapid CARB	1 (0.9%)	8
Automated system Phoenix™ AST/ID	7 (6.1%)	20, 112, 37, 13, 88, 108, 109
MALDI-TOF (mass spectrometry)	8 (7%)	14, 22, 31, 46, 70, 71, 86, 90
Molecular detection method		
PCR assay (conventional PCR, multiplex PCR)	46 (40.4%)	2, 9,17, 18, 20, 22, 25–32, 34, 36, 39–41, 47, 49–51, 54, 55, 57, 58, 62, 63, 67–72, 86, 95–97, 101, 109–114
RT-qPCR	5 (4.4%)	14, 48, 51, 71, 104
ERIC-PCR (or rep-PCR, box PCR)	5 (4.4%)	51, 25, 28, 64, 81
pulse field gel electrophoresis (PFGE)	7 (6.1%)	7, 8, 15, 38, 79, 89, 97
multilocus sequence typing (MLST)	7 (6.1%)	6, 10, 63, 96, 97, 106, 107
Sequencing by Sanger ABI 3730/ ABI PRISM®3500, whole genome sequencing (WGS)/ Illumina sequencing	13 (11.4%)	26, 37, 44, 47, 57, 58, 64, 72, 89, 104, 108, 109

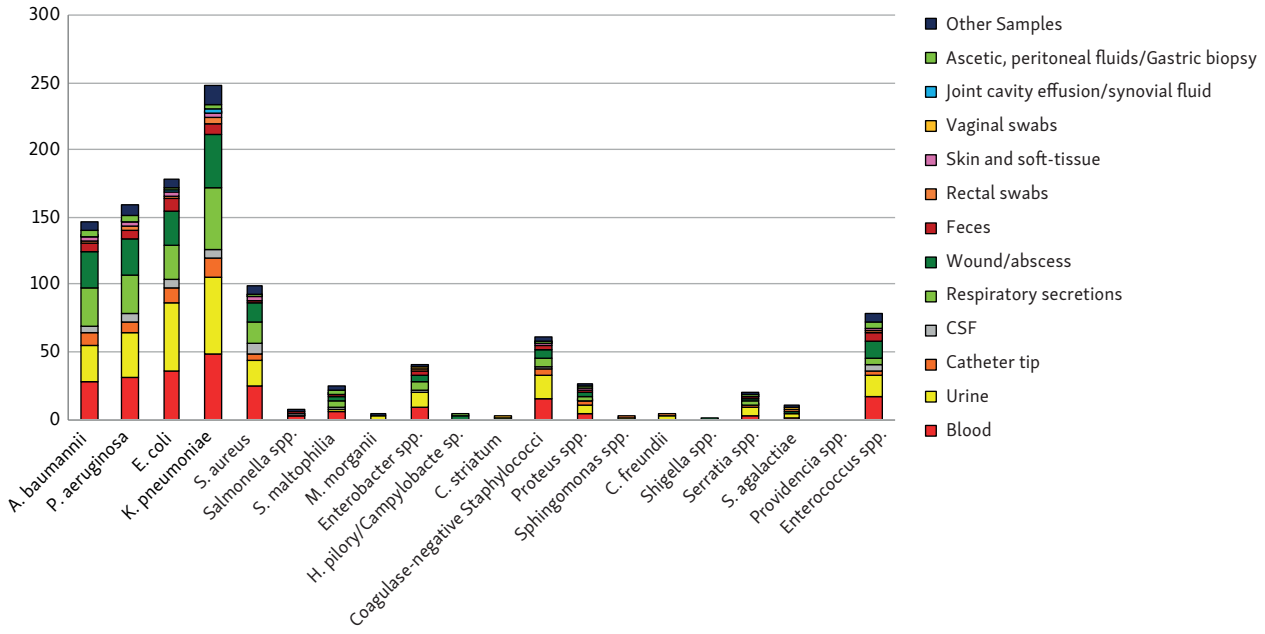

Fig. 2 Distribution of bacterial species between different clinical samples.

most of them presented often resistance to fluoroquinolones, ampicillin, co-trimoxazole and cephalosporins (3, 4, 11, 17, 21, 26, 33, 39, 54, 62, 64, 73, 85, 110). Moolchandani et al., recommends not using these antibiotics for empirical therapy of urinary tract infections acquired in ICUs in South India; instead, they suggest considering imipenem, pipericillin-tazobactam, amikacin, and nitrofurantoin for initial therapy with prompt de-escalation after culture and sensitivity results are received (3).

K. pneumoniae was also the most reported in blood samples, respiratory secretions, and swabs from wounds (n = 49, 46, 40, respectively). In blood samples, *E. coli, Pseudomonas aeruginosa,* and *Acinetobacter baumannii* were reported in 36, 31, and 28 articles, respectively. An important feature among these Gram-negative bacteria was the production of extended-spectrum β-lactamases (ESBL) and carbapenemase.

Among Gram-positive bacteria, Methicillin-Resistant *Staphylococcus aureus* (MRSA) was the most reported in blood and urine samples in 25 and 19 studies, respectively, followed by Vancomycin-Resistant *Enterococcus* (VRE) in 17 and 15 studies, respectively. Urine samples from which the MRSA was isolated corresponded to a urine catheter positioned in the bladder or in the ureter (2, 7, 13, 19, 49, 59, 82, 84, 93, 108).

There are a large number of studies reporting MDR pathogens in different parts of the world, which would explain the factors that trigger the increase in epidemic outbreaks, morbidity and mortality, with significant direct and indirect costs (8, 10, 11, 12, 15, 17, 29, 34, 37, 50, 62, 65, 68, 87, 91). The most frequently reported MDR microorganisms in this last decade were found among isolates of *K. pneuomaniae, E. coli, P. aeruginosa, A. baumannii*, SARM and VRE. The number of reports of MDR microorganisms varied geographically, with the highest number of reports being made in Asia (25 studies) and the lowest number being in North America (3 studies). These differences occur not only between continents, but even between countries, with the highest number of reports recorded in Egypt (in 8 studies) and Iran (in 7 studies). Infection in elderly patients, long duration of hospitalisation, use of broad-spectrum antibiotics and long-term or continuous use of a single antibiotic have been recognize as risk factors for development of infection caused by MDR pathogens as suggested by Buetti et al. (16).

Hypervirulent *K. pneumoniae* (hvKp) is an emerging pathotype that is more virulent than classical *K. pneumoniae.* hvKp carry plasmids with genes that code for a large number of virulence factors (such as the capsule that protects bacteria from both phagocytosis and lethal serum factors, fimbriae, lipopolysaccharides and siderophores) and resistance to heavy metals (copper, silver, lead and tellurite) (27, 46, 106). Although hvKp strains are usually susceptible to most antimicrobials, an increased prevalence of MDR-hvKp nosocomial strains, including carbapenemase-producing strains has already been described, mainly in patients with healthcare-associated infections in Egypt (27, 114), India (44), Iran (46), and China (101). Further limiting the range of therapeutic alternatives, since the dissemination of a hypervirulent strain in hospitalized patients could have serious consequences, it is recommended to implement contact precautions against suspicion.

Another important aspect found in this review was the report of *Stenotrophomonas maltophilia* and *Corynebacterium striatum*, which have been reported in recent years among the group of MDR opportunistic pathogen as a cause of infection particularly among hospitalized patients.

S. maltophilia is an opportunistic pathogen that has high intrinsic and acquired antimicrobial resistance, among the therapeutic options to treat infections due to MDR-*S. maltophilia* is trimethoprim-sulfamethoxazole. However, some strains resistant to this antibiotic are

already reported with prevalences between 2.4% and 10.7% in hospitals in Egypt (29), China (43), Iran (47, 48), North America (74, 95), and Mexico (83).

C. striatum is considered a normal component of the human skin and mucosal microbiota, however, it is frequently cited as a pathogen of hospital-acquired infections in some hospitals in Tunisia (76) and China (86). A high prevalence of MDR-*C. striatum* isolates (>50%) was reported in these hospitals, supporting the idea that it is an emerging MDR-bacterium.

DISTRIBUTION OF ANTIBIOTIC RESISTANCE GENES (ARG)

A total of 50 types of ARG were found in this systematic review. Asian hospitals present bacterial isolates with the greatest diversity of detected ARGs, followed by Africa, Europa, Latin America and North America. The highest ARG diversity was reported in bacteria that were causing hospital-acquired infections from Asia and Africa

In Asia, 80 ARGs were reported, distributed in 31 types, including *bla* (conferring resistance to β-lactam antibiotics) (27.5%), *aac* (cause resistance to aminoglycosides) (8.8%), and *tet* (cause resistance to tetracyclines (5%). In Africa, 47 ARGs distributed in 22 types are reported, *bla* gene was reported in 30.4%, followed by the *aac* gene with 8.7%. However, in some bacteria the mechanism of resistance to antibiotics is mainly mediated by chromosomal mutations, as is the case of *C. striatum,* all quinolone-resistant isolates showed mutations in the *gyrA* gene as reported in hospitals in Tunisian (76) and China (86).

Studies in Europe reported 24 types of ARGs with a higher abundance of *bla* genes (17.1%), followed by genes: *acc*, *mph* (cause resistance to macrolide), *qepA* (encodes an efflux pump that reduces susceptibility to fluoroquinolone), *sul* (cause resistance to sulfonamides), *aad* (cause resistance to aminoglycosides), *aph* (cause resistance to streptomycin), and *ddl* (mutations in this gene confer D-cycloserine resistance) (5.7% each), while in Latin America, of the 15 types of ARGs found in this review, 20.8% correspond to the *bla* genes followed by *acc, aph, sul, tet,* and *mcr* (cause resistance to colistin) (8.3% each). Although only 2 types of ARGs were reported in North America, they present greater abundance compared to reports in other parts of the world, the *bla* gene represented 83% and *van*A/B (cause resistance to vancomycin) (16.7%).

The highest number of ARGs (n = 24) was detected in *S. aureus*, followed by *K. pneumoniae* (20 ARGs), *A. baumannii* (16 ARGs), and *E. coli* (14 ARGs) (Fig. 3).

The *bla* genes were reported in 53 studies and distributed in 11 bacterial species, representing 46.5% of the AGRs found in this systematic review. *K. pneumoniae* was the most reported with *bla* genes (28 studies), followed by *E. coli* (21 studies), and *P. aeruginosa* (12 studies). The *bla* genes were also detected in other emerging MDR organisms, such as *C. striatum*, all penicillin resistant isolates were positive for the *bla* gene in Tunisian hospitals (76).

The *bla*OXA subtype (cause production of oxacillinases and resistance to *β-lactam* antibiotics, including carbapenems) is the most reported in this group (30 studies), followed by *bla*CTX (cause production of cefotaxime-hydrolyzing *β-lactamase* and resistance to *β-lactam* antibiotics,

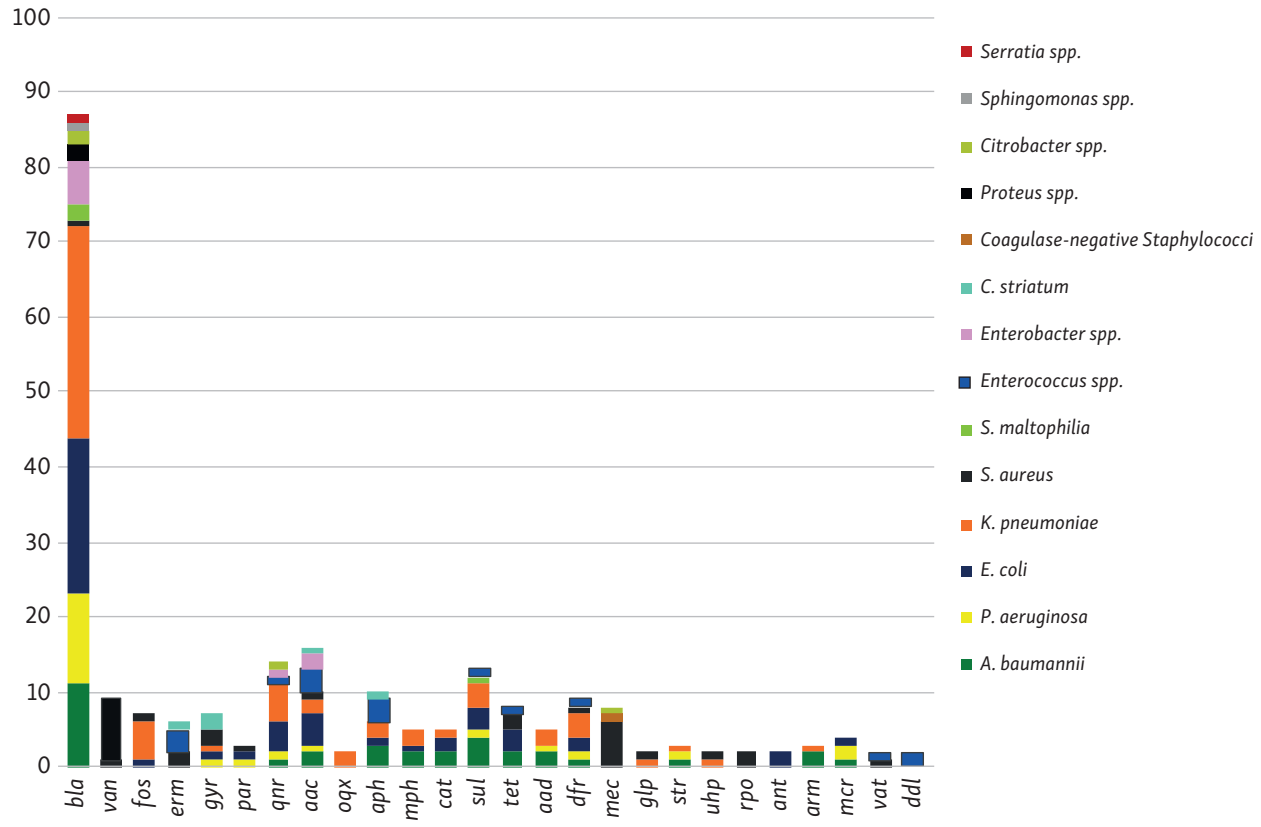

Fig. 3 Abundance and diversity of antibiotic resistance genes (ARG) in individual bacteria.

especially cefotaxime and ceftriaxone) (28 studies) and *bla*TEM (cause production of narrow-spectrum β-lactamases and resistance to to penicillins and early cephalosporins) (25 studies). The ARGs *aac* was reported in 15 studies (13.2%) and *sul* in ten studies (8.8%). However, resistance to aminoglycosides presented the greatest diversity of ARGs (*aac, smeD/F, aad, ant, arm rmt, aph, msr*).

Plasmids and others active mobile elements such as transposons and integrons are horizontal gene transfer vehicles, that give bacteria great capacity to adapt to changes in the environment. These mobile elements play a crucial role in the dissemination of ARGs in populations of pathogenic bacteria, favoring multiresistance. The most frequent antibiotic resistance genes, such as genes coding the production of ESBL, are located in plasmids. Recent studies point to plasmid-mediated transfer in hospitals in Africa (17, 19, 25–28, 33, 52, 101), Asia (35, 40, 42, 45, 57, 94, 98, 105), Latin America (63–65), Europe (67, 72, 91), and North America (89, 100, 103).

Other types of ARGs located on plasmids have been reported, such as the *mcr-1* gene they have been detected in isolates of *A. baumannii* and *P. aeruginosa* resistant to colistin (41, 58, 97). The *aac* and *ant* genes responsible for aminoglycosides resistance were detected in isolates of *K. pneumoniae* (19), VRE (20, 36), *S. maltophilia* (48) and *E. coli* (50), and *fos* genes, which confer resistance to fosfomycin, have been reported on plasmids and active mobile genetic elements of *E. coli* (54), *K. pneumoniae* (79, 54, 56, 72, 104) and MRSA (59, 96).

Next type of active mobile elements such as transposons and integrons have also been shown to be very efficient in the propagation of AGRs in bacteria that cause infections in the ICU. In MDR *A. baumannii*, the transportable elements, Tn2006, Tn2007, Tn2008, and Tn2009, play a key role in the transfer of the *bla*OXA-23 gene. Isolates with Tn2006 has been detected in predominantly in Iran (113), while Tn2008, and Tn2009 in China (90, 106). Also, high frequency of MDR pathogens harboring class 1 and 2 integrons have been detected in *K. pneumoniae* (9, 14), *A. baumannii* (10, 65, 57, 77, 90, 113), *P. aeruginosa* (18, 94, 101), and *E. coli* (38, 64, 110).

GENETIC DIVERSITY OF ANTIBIOTIC RESISTANT ISOLATES

Bacteria that cause hospital-acquired infections are chracterised by a genetic structure composed of a high genotypic diversity, but a predominance of several clones can be found. Whole genome analysis (WGS)-based analysis on MDR and ESBL-positive *E. coli* evidenced high genetic diversity in hospitals in Benin (22) and Bangladesh (35). However, a study conducted in Mozambique using ERIC-PCR analysis revealed that despite evidence of high genetic diversity among *E. coli* isolates, there was a predominance of few clones adapted to the hospital environment, what would they probably be HiRCs (17). Similar findings were reported in hospitals in Ethiopia (25) and Colombia (64). Analysis by pulsed field gel electrophoresis (PFGE, technique used to produce a DNA fingerprint for a bacterial isolate) also supports these findings: among the great diversity of pulse types (ST), ST405 and ST1284 circulate mainly in hospitals in Lebanon (38), while ST131 in Bangladesh (35) and USA (89, 100).

The genetic structure of *A. baumannii* shows a similar behavior. MLST analysis performed on clinical isolates of carbapenem-resistant *A. baumannii* identified carriers of *bla*OXA-23, belonging to ST2 circulating in hospital settings in South Africa (77), and ST195, ST540, and ST208 in China (90).

The phylogenetic analysis using WGS in *A. baumannii* showed that all isolates analyzed in a hospital in Iran belonged to the same clade, within lineage 2 of global clone1 (113).

The population structure of *K. pneumoniae* is more heterogeneous than that observed in isolates of *E. coli* and *A. baumannii,* which emphasizes the opportunistic nature of these species. The results obtained among KPC producing *K. pneumoniae* also reflect the well-known dominance of ST258 clone in USA (100). Multilocus sequence typing in carbapenem-resistant *K. pneumoniae* strains showed that ST15 was prevalent in Portugal (4), ST395 in France (69), ST11 in China (106, 107), and ST14, ST5188, ST1861 in Iran (98).

The GWAS analysis that was performed on KPC-producing *K. pneumoniae* isolates from epidemic outbreaks in hospitals in Switzerland during 2013 and 2015 revealed low variability among isolates, contrary to the results given by plasmid analysis. Each epidemic outbreak was dominated by clone ST512, which was probably adapted to the antibiotic therapy used at the time (72).

GWAS analysis was also performed on HvKp strains obtained from hospital-acquired infections in Indian, and showed that these strains evolved in few clones (ST23, ST240, and ST2319 (44). The study by Sanikhani et al, in two Iranian teaching hospitals also detected clone ST23 in all hvKp isolates (46).

The number of carbapenemase-producing *P. aeruginosa* strains has also been increasing in medical settings in ICUs (18, 24, 28, 32, 43, 101). ST1816 has emerged and evolved in the medical environment of Japan (99), and ST260 is the most frequent in hospitals in USA and Estonia (5, 91, respectively), mostly with a MDR phenotype.

In relation to Gram-positive pathogens, it is reported that MRSA strains are leading causes of hospital-acquired infections in the United States, and clonal complex 5 (CC5) is the predominant lineage responsible for these infections (74). ST772-t657 is the most reported MRSA clone in tertiary hospitals in Pakistan (59), and ST239-t030 is detected in all cases of hospital-acquired infections in Yunnan Province of China, it belongs to 'Turkish clade' from Eastern Europe (96). Genetic relatedness of MDR-*E. faecium* isolates in university hospitals in Serbia was established by Multiple-locus variable-number tandem-repeat (VNTR) analysis (MLVA), which revealed polyclonal setting with 25 unique MT profiles, which are either single-locus or double-locus variants of clones MT-340 and MT-159, known to cause infections in hospitalizied patients in Serbia. These are isolates that have most likely been selected by antibiotic pressure and develop in hospital-adapted clones that occur sporadically (109). Using PFGE analysis, Kohler *et al*. demonstrated a high clonality in strains of *Enterococcus spp.* causing bacteremia in several Canadian ICUs (112).

Among the mechanisms to control problematic pathogens in ICUs, some authors propose implementing close surveillance and detection of resistant pathogens, changes in resistance pattern, s as well as applying strict cleaning protocols, antibiotic administration policies and adequate control guidelines to the specific conditions for each hospital (5, 7, 8, 13, 15, 24, 66, 81).

Our study provides information on the epidemiological behavior of pathogens that cause infections in adult ICUs. Disadvantage of our study is that the studies used for the analysis were heterogeneous and some studies did not report ARGs or did not perform genetic diversity analyses. There were very few reports that used state-of-the-art molecular techniques to carry out the analysis of the genetic structure of bacteria isolated from nosocomial infections.

CONCLUSIONS

In this systematic review it is evident that *K. pneumoniae* and *E. coli* were the most reported in urinary tract infections, bacteremia and pneumonia in hospitals in Asia, Africa and Latin America, being the production of ESBL and carbapenemases mediated by *bla*OXA *and bla*CTX genes, the mechanism of resistance most common in these bacteria. However, it is evident that there are important differences between regions, such as the reports of *P. aeruginosa* in Europe and North America as the second most prevalent pathogen after *K. pneumoniae* or *E. coli,* respectively. The main concerns about MDR-pathogens are usually associated with gram-negative bacilli, ESBL, and carbapenemase-producing strains of *E. coli* and *K. pneumoniae*, as well as carbapenemase-producing *P. aeruginosa* and *A. baumannii*. Among gram-positive nosocomial pathogens, MRSA and VRE are often reported. In some ICUs around the world there is a marked presence of MDR, XDR and PDR organisms, shows great diversity, probably due to the selective action exerted by the use of intensive empirical antibiotic therapy. However, there is a predominance of few clones that have adapted efficiently to the hospital environment: mainly CC5 MRSA strains are leading causes of hospital-acquired infections in the United States (74). Clone ST23 KPC-producing *K. pneumoniae* is isolated from infections in India (46) and Iran (47) and ST260 carbapenemase-producing *P. aeruginosa* is the most frequent in hospitals in United States (85) and Estonia (91) and have a great ability to survive for a long time. These are the high-risk clones that must be closely monitored due to their spread ant to the greater capacity to cause additional morbidity, mortality, and hospital costs.

CONFLICTS OF INTEREST

The author declares that there are no conflicts of interest.

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Wireless Monitoring of Gastrointestinal Transit Time, Intra-luminal pH, Pressure and Temperature in Experimental Pigs: A Pilot Study

Jan Bureš[1,2,3,*], Věra Radochová[4], Jaroslav Květina[3], Darina Kohoutová[3,5], Martin Vališ[6], Stanislav Rejchrt[7], Jana Žďárová Karasová[8], Ondřej Soukup[3], Štěpán Suchánek[1,2], Miroslav Zavoral[1,2]

ABSTRACT

Background: There is no single gold standard for investigation of gastrointestinal motility function. Wireless motility monitoring involves a novel concept which provides a complex information on gastrointestinal function (gastrointestinal transit time, intra-luminal pH, pressure and temperature). Gastrointestinal motility functions of experimental pigs are very similar to those of humans. That is why porcine studies have already provided suitable experimental models for several preclinical projects.

Aims: The aim of our study was to adopt methods of non-invasive wireless monitoring of gastrointestinal functions in experimental pigs.

Methods: Five experimental adult female pigs were enrolled into the study. Wireless motility capsules were delivered into the porcine stomach endoscopically. Gastrointestinal transit and intra-luminal conditions were recorded for five days.

Results: Records of animals provided good (3 pigs) or very good quality files (2 pigs). 31150 variables were evaluated. Mean time of the presence of capsules in the stomach was 926 ± 295 min, transfer of a capsule from the stomach into the duodenum lasted 5–34 min. Mean small intestinal transit time was 251 ± 43 min. Food intake was associated with an increase of gastric luminal temperature and a decrease of intra-gastric pressure. The highest intra-luminal pH was present in the ileum. The highest temperature and the lowest intra-luminal pressure were found in the colon. All data displayed a substantial inter-individual variability.

Conclusions: This pilot study has proven that a long-term function monitoring of the gastrointestinal tract by means of wireless motility capsules in experimental pigs is feasible. However, both ketamine-based induction of general anaesthesia as well as long-lasting general anaesthesia (> 6 hours) should be avoided to prevent retention of a capsule in the porcine stomach.

KEYWORDS

acetylcholinesterase inhibitors; experimental pigs; gastrointestinal transit time; intra-luminal pH; pressure and temperature; oncology; toxicology; wireless capsule monitoring

AUTHOR AFFILIATIONS

[1] Institute of Gastrointestinal Oncology, Military University Hospital Praha, Czech Republic
[2] Department of Medicine, Charles University, First Faculty of Medicine, Praha and Military University Hospital Praha, Czech Republic
[3] Biomedical Research Centre, University Hospital Hradec Králové, Czech Republic
[4] Animal Laboratory, University of Defence, Faculty of Military Health Sciences, Hradec Králové, Czech Republic
[5] The Royal Marsden Hospital NHS Foundation Trust, London, United Kingdom
[6] Department of Neurology, Charles University, Faculty of Medicine in Hradec Králové and University Hospital Hradec Králové, Czech Republic
[7] 2nd Department of Medicine – Gastroenterology, Charles University, Faculty of Medicine in Hradec Králové and University Hospital Hradec Králové, Czech Republic
[8] Department of Toxicology and Military Pharmacy, University of Defence, Faculty of Military Health Sciences, Hradec Králové, Czech Republic
* Corresponding author: Institute of Gastrointestinal Oncology, Military University Hospital Praha, U Vojenské nemocnice 1200, 169 02 Praha 6, Czech Republic; e-mail: bures.jan@uvn.cz

Received: 1 January 2023
Accepted: 21 March 2023
Published online: 26 June 2023

Acta Medica (Hradec Králové) 2023; 66(1): 11–18
https://doi.org/10.14712/18059694.2023.9

INTRODUCTION

Motility function of the entire gastrointestinal tract belongs to the most complex and most fragile systems in human body (1). There is no single gold standard for its investigation. Usually, it is necessary to combine several methods. Motor function of the oesophagus is mostly investigated by means of oesophageal impedance/pH-metry, reflux scintigraphy and/or high-resolution manometry (2–7). Examination of the gastric motility function uses gastric emptying scintigraphy, ^{13}C-acetate or ^{13}C-octanoic acid breath tests, electrogastrography, magnetic resonance imaging, antroduodenal manometry, ancillary testing (including barostat and satiety testing) or EndoFLIP (Endoscopic Functional Lumen Imaging Probe: high-resolution impedance planimetry system). EndoFLIP may be also used for the assessment of oesophageal motility function, in fact, it can replace oesophageal manometry in some patients (8–18). Investigation of the intestinal motor function is the most demanding and the least accurate. There are only a few methods available for routine clinical practice so far. Examinations usually rely on the oro-caecal, small intestinal and/or colonic transit time measurements (lactose-(13C)ureide breath test, indirectly hydrogen & methane breath tests, scintigraphic transit time testing, radio-opaque markers and pellets propulsion) (19–21). Synucleinopathy is an indirect marker of motor dysfunction (e.g. in Parkinson disease) (22). Important part of a complex investigation is small intestinal bacterial overgrowth testing (23–25).

Wireless motility monitoring involves a novel concept which provides a complex information on gastrointestinal function. The wireless motility/pH capsule is an orally ingested, non-digestible, data recording device that enables a simultaneous assessment of a segmental and whole gut transit. This capsule was approved by the US Food and Drug Administration for the evaluation of patients with suspected delayed gastric emptying and the evaluation of colonic transit time in patients with chronic idiopathic constipation. The device continuously measures temperature, pH, and pressure of its surrounding structures while passing through the gastrointestinal tract (via gut peristalsis) until exiting the body through the anus. Validated patterns in pH and temperature recordings allow accurate measurements of gastric emptying, small bowel transit, colonic transit, and whole gut transit times (26–28).

Gastrointestinal motility functions of experimental pigs are very similar to those of humans. That is why porcine studies have already provided suitable experimental models for several preclinical projects (29–31). Group of Professor Griffin published an important study of wireless recording of the gastrointestinal motility and luminal conditions in experimental pigs (32). The aim of our current study was to adopt Professor Griffin's methods to our own porcine experimental setting. Studies on gastrointestinal motility are of utmost importance, especially in the context of side effects induced by drugs, e.g. medication used for treatment of dementia and malignancies.

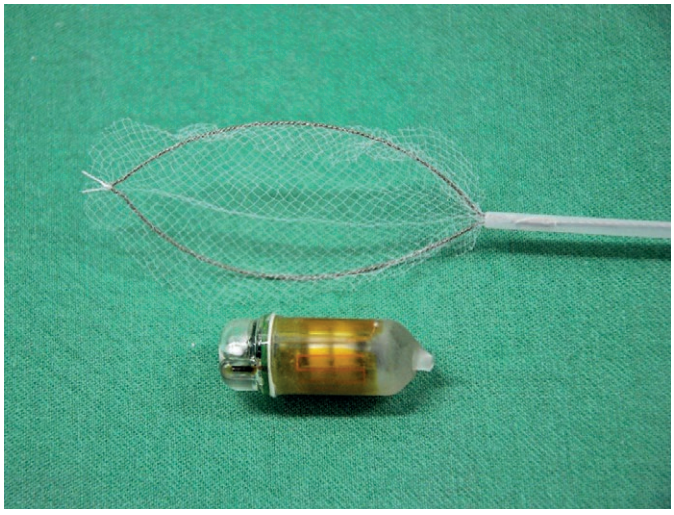

Fig. 1 Wireless motility capsule measures 27 × 12 mm (weight 4.5 g). A special roth net was used for capsule delivery into the porcine stomach.

METHODS

PRELIMINARY TESTING

Preliminary data were obtained from three experimental adult female pigs (*Sus scrofa* f. *domestica*, hybrids of Czech White and Landrace breeds; 4-month-old; mean weight 40.2 ± 1.5 kg; median 39.5 kg). The aim of this preliminary part was planned to check the feasibility of wireless data acquisition. However, both ketamine-based induction of anaesthesia (20 mg/kg i.m.) in one animal and long-term general anaesthesia (> 6 hours) in another two pigs were associated with a retention of motility capsules in the porcine stomach during the entire 5-day period.

ANIMALS

Another five experimental adult female pigs (*Sus scrofa* f. *domestica*, hybrids of Czech White and Landrace breeds; 4-month-old; mean weight 41.2 ± 5.5 kg; median 39.5 kg) were enrolled into the main part of the study. The animals were purchased from a certified breeder (Štěpánek, Dolní Ředice, Czech Republic; SHR MUHO 2050/2008/41). The pigs were housed in an accredited animal laboratory (Faculty of Military Health Sciences, Hradec Králové). During a two-week acclimatization, all animals were fed with a standard assorted A1 food (Ryhos, Nový Rychnov, Czech Republic) in equal amounts twice a day, and had free access to a drinking water.

DESIGN OF THE STUDY

All experiments were commenced in the morning on overnight fasting animals. Drugs used as an induction of anaesthesia were medetomidine 0.1 mg/kg i.m., butorphanol 0.3 mg/kg i.m. and midazolam 0.3 mg/kg i.m. Subsequent short-term general anaesthesia was maintained by i.v. propofol (repeated one-mL boluses per 20 mg, in total less than 5 mL; time < 10 min.) only for the endoscopic delivery of motility capsules into the middle part of the gastric body.

Wireless motility capsules (SmartPill, Medtronic, Dublin, Ireland) were purchased from Imedex (Hradec Králové, Czech Republic). Capsules were delivered into the porcine stomach endoscopically using a video-gastroscope GIF-Q180 (Olympus Optical Co, Tokyo, Japan) dedicated for animal use only. A special roth net (Steris, US Endoscopy, Mentor, OH, USA) was used to facilitate this installation (Figure 1). After full recovery from a short-term general anaesthesia, animals were free to move in unlimited manner with an unrestricted access to water. Food intake was allowed from four hours onwards. Data from wireless motility capsules was recorded for five days continuously, and these were available for subsequent detailed analysis.

STATISTICS

All data was tested statistically by means of the SigmaStat software (Version 3.1, Jandel Corp, Erkrath, Germany). Distribution of data was assessed by Kolmogorov-Smirnov test; Shapiro-Wilko test was used for evaluation of normality of sampled data. Descriptive statistics, unpaired t-test (for normal distribution) and Mann-Whitney rank sum test (for non-normal distribution) were used to treat variables.

ETHICS

The Project was approved by the Institutional Review Board of the Animal Care Committee of the University of Defence (Protocol Number MO 171673/2019-684800), Faculty of Military Health Sciences, Hradec Králové. The study was conducted in accordance with the policy for experimental and clinical studies (33). Animals were held and treated in conformity with the European Convention for the Protection of Vertebrate Animals (34).

RESULTS

Records of animals of the main study part provided good (3 pigs) or very good quality files (2 pigs), see Figure 2. Mean time of overall recording was 6537 ± 712 min. (median 6538 min.). In total, 31150 variables were evaluated. Mean time of the presence of capsules in the stomach was 926 ± 295 min. (median 1091 min.), transfer of a capsule from the stomach into the duodenum lasted 5–34 min. (median 8 min.). Capsules migrated back from the duodenum into the stomach spontaneously three times (for 13, 14 and 63 min.). Mean small intestinal transit time was 251 ± 43 min. (median 233 min.). Other major results are shown in Table 1 and Figures 3–6.

DISCUSSION

We have implemented investigation of gastrointestinal motility function by means of wireless capsules to our experimental practice successfully. Yet, our initial experience is still limited, therefore it is required to evaluate our first findings with caution.

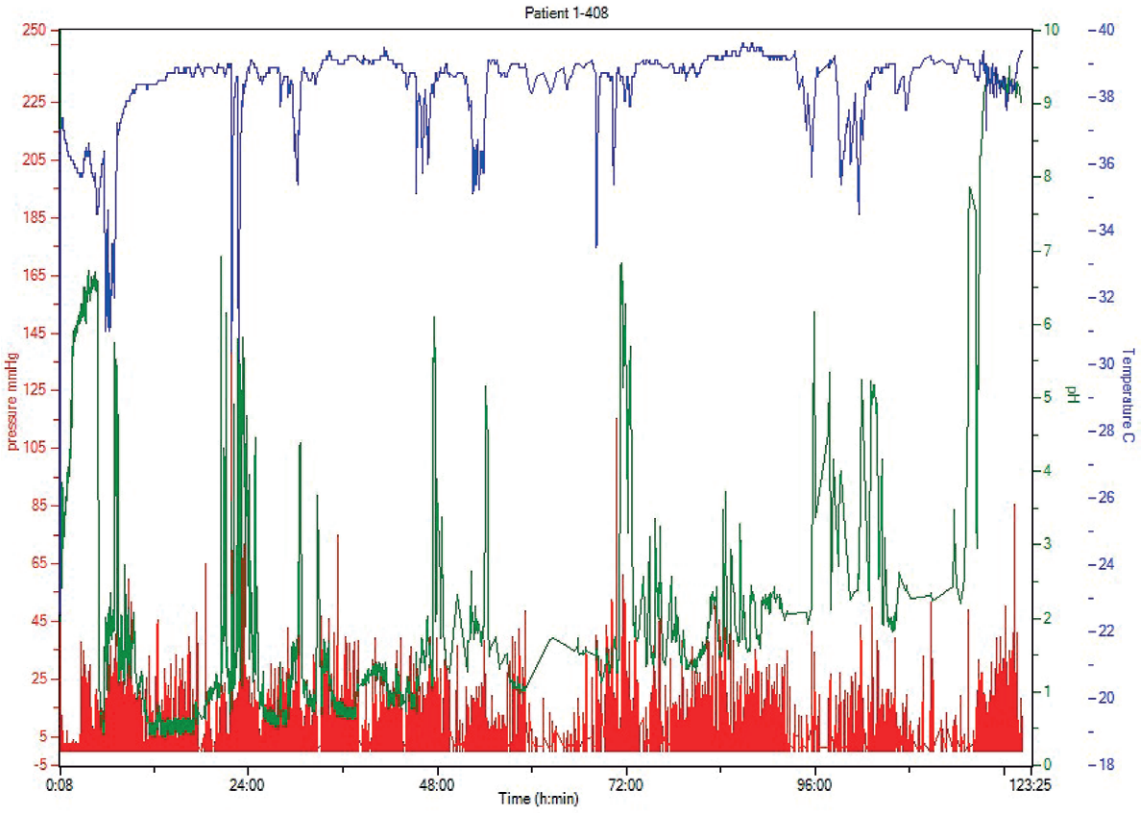

Fig. 2 Intra-luminal pressure is recorded 120-times per minute (mm Hg), pH is registered 12-times per minute and temperature is measured 3-times per minute (°C). Pressure in red, pH in green, temperature in blue.

Tab. 1 Wireless monitoring of intra-luminal pH, temperature and pressure.

Parameter	A Mean ± Std. Dev. Median IQR	B Mean ± Std. Dev. Median IQR	C Mean ± Std. Dev. Median IQR	D Mean ± Std. Dev. Median IQR	E Mean ± Std. Dev. Median IQR	F Mean ± Std. Dev. Median IQR	Relevant significance
pH	1.1 ± 0.6 1.1 0.6–1.4	4.5 ± 0.7 4.6 4.3–4.9	4.4 ± 1.5 4.7 3.1–5.2	8.1 ± 1.3 8.4 7.8–8.8	9.0 ± 0.2 8.9 8.9–9.1	7.5 ± 0.5 7.3 7.1–7.8	B > A (p < 0.001) E > D (p < 0.001) E > F (p<0.001)
Temperature	38.8 ± 1.4 39.3 38.9–39.5	39.2 ± 0.4 39.3 39.1–39.4	39.1 ± 0.2 39.1 39.1–39.3	39.1 ± 0.6 39.1 38.8–39.6	39.2 ± 0.7 39.4 38.5–39.8	40.0 ± 0.4 40.0 39.6–40.3	B > A (p = 0.004) F > D (p < 0.001) F > E (p < 0.001)
Pressure	2.4 ± 1.3 2.1 1.5–2.8	2.1 ± 1.6 1.5 1.1–2.5	3.5 ± 3.7 2.3 1.5–3.8	3.6 ± 2.2 3.2 2.5–4.2	4.1 ± 2.4 3.6 2.7–4.8	2.1 ± 1.6 1.6 1.0–2.6	A > B (p < 0.001) C > B (p < 0.001) E > D (p < 0.001) E > F (p < 0.001)
Maximal pressure	10.4 ± 11.1 7.0 3.8–13.7	11.3 ± 13.6 6.8 3.1–16.1	27.9 ± 64.7 9.9 3.2–17.3	18.4 ± 30.4 14.1 9.7–18.9	19.3 ± 13.5 17.3 11.3–23.3	10.1 ± 9.8 7.7 3.1–14.3	A > B (p = 0.018) C > B (p = 0.014) E > D (p < 0.001) E > F (p < 0.001)

Group A: stomach under fasting condition; B: stomach after food intake; C: passage of a capsule from the stomach to the duodenum; D: jejunum; E: ileum; F: colon. The pH inversely indicates the concentration of hydrogen ions in the solution; values of temperature are given in degrees Celsius (°C); values of pressure are measured in Torr (mm Hg).
pH = −log(H+) = −log([H+]/M); Std. Dev.: standard deviation; IQR: inter-quartile range

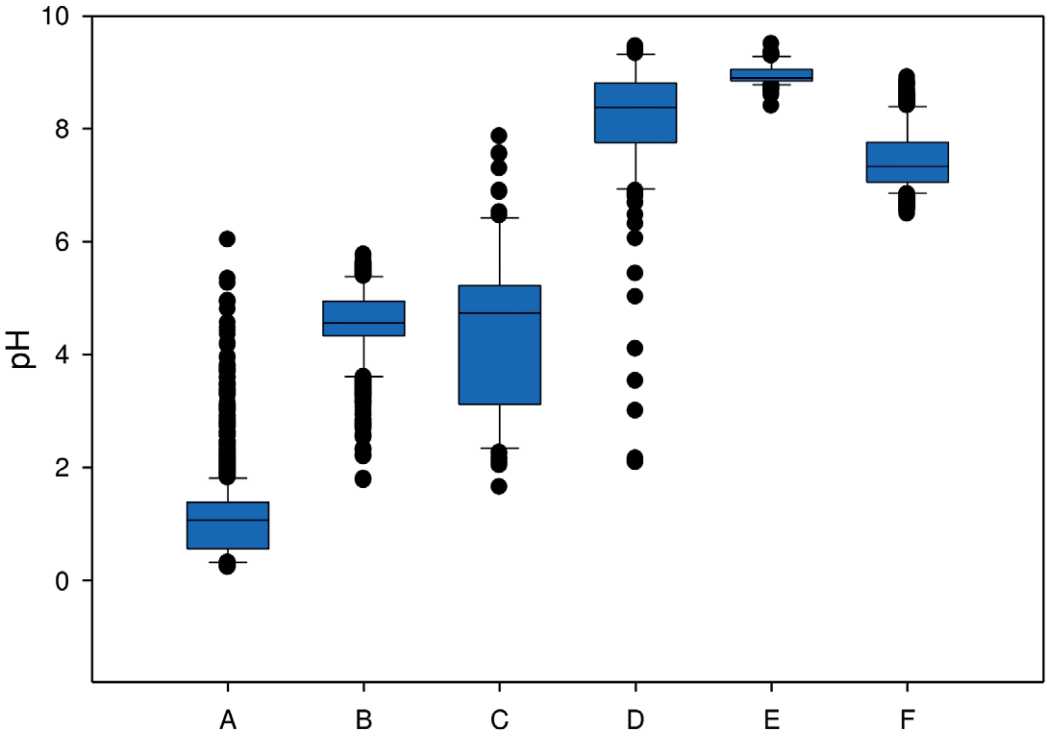

Fig. 3 Monitoring of intra-luminal pH. Group A: stomach under fasting condition; B: stomach after food intake; C: passage of a capsule from the stomach to the duodenum; D: jejunum; E: ileum; F: colon. Median, inter-quartile range and outliers. Selected statistically significant differences: B > A (p < 0.001); E > D (p < 0.001); E > F (p < 0.001).

To highlight our results, the presence of capsules in the stomach was surprisingly long. We assume that this time does reflect rather delayed expulsion of a capsule from the porcine stomach than real gastric emptying time. Nevertheless, according to our previous endoscopic experience, the porcine stomach has almost always contained remnants of food, even after a long fasting period. Torus pyloricus (muscular gatekeeper of the pylorus) contributes to this fact. Food intake was associated with an increase of gastric luminal temperature and a decrease of intra-gastric pressure. Total small intestinal transit time was relatively short (entire length of the porcine small bowel is around 12 metres). The highest intra-luminal pH was present in the ileum. The highest temperature and the lowest intra-luminal pressure were found in the colon. All data displayed a substantial inter-individual variability.

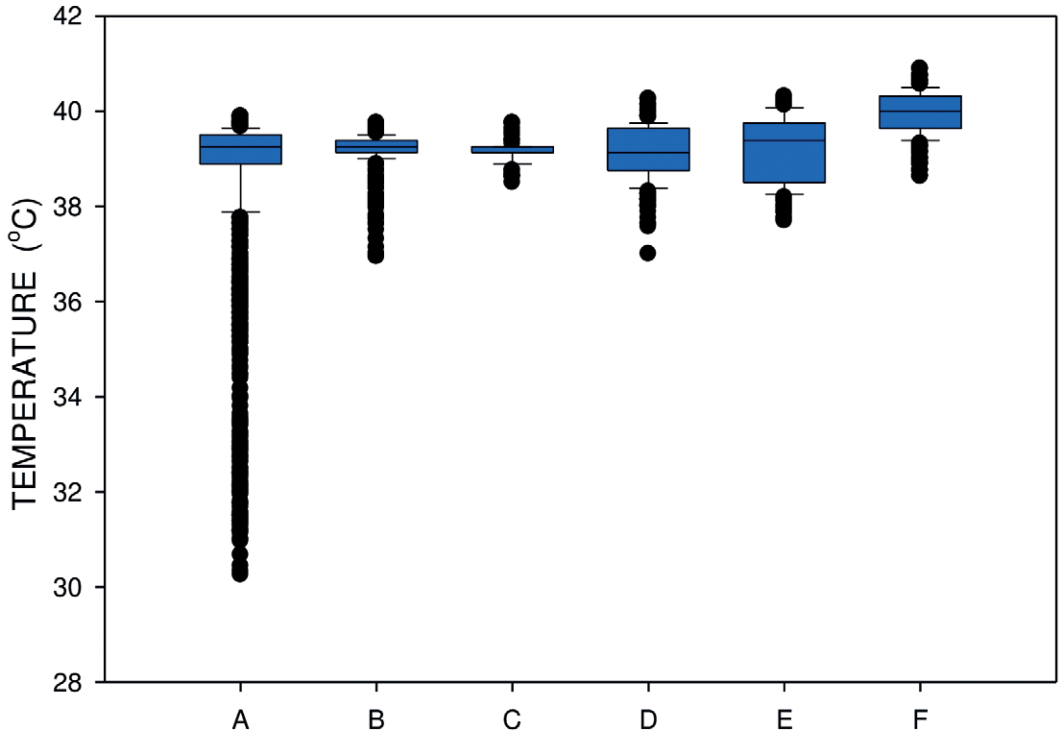

Fig. 4 Intra-luminal temperature. Group A: stomach under fasting condition; B: stomach after food intake; C: passage of a capsule from the stomach to the duodenum; D: jejunum; E: ileum; F: colon. Median, inter-quartile range and outliers. Selected statistically significant differences: B > A ($p = 0.004$); F > D ($p < 0.001$); F > E ($p < 0.001$).

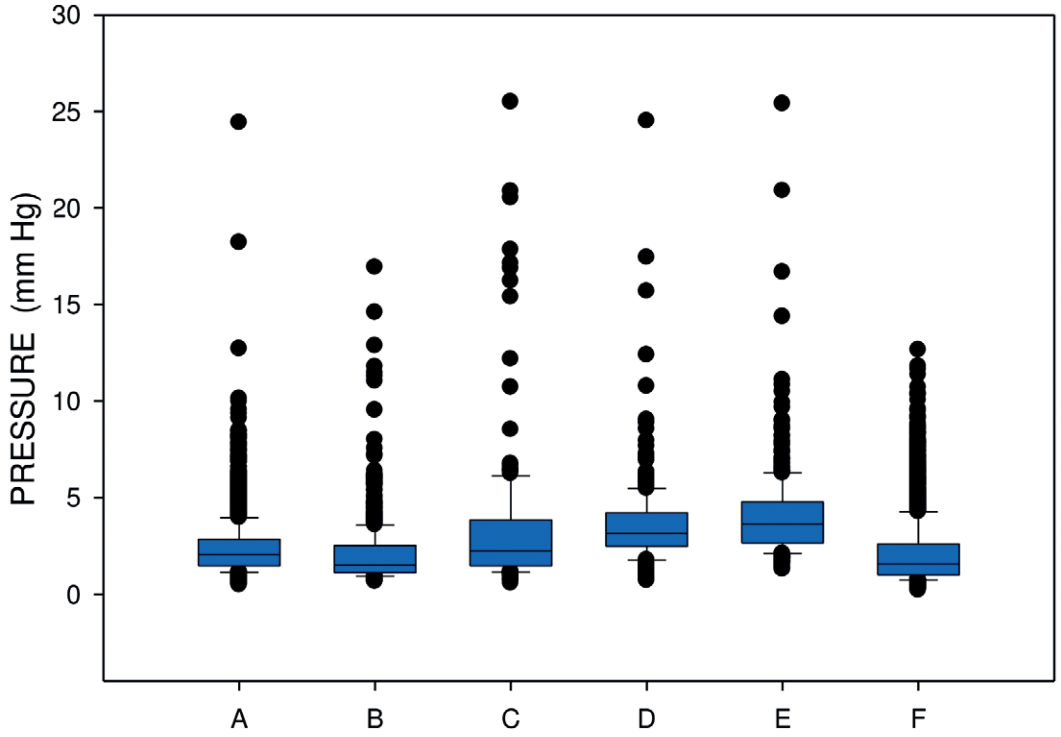

Fig. 5 Intra-luminal pressure. Group A: stomach under fasting condition; B: stomach after food intake; C: passage of a capsule from the stomach to the duodenum; D: jejunum; E: ileum; F: colon. Median, inter-quartile range and outliers. Selected statistically significant differences: A > B ($p < 0.001$); C > B ($p < 0.001$); E > D ($p < 0.001$); E > F ($p < 0.001$).

Similar variability features were found in experimental porcine electrogastrography, too (35–38). In this study, we decided to deliver the wireless capsule endoscopically, so that gastric content could be removed from the stomach before the capsule was placed (drunk water, gastric juice, regurgitated bile). Endoscopy also enabled a quick insertion of a capsule and thus shortened the duration of general anaesthesia.

There are only sparse experimental studies available so far (32, 39–41). The most detailed data on the use of

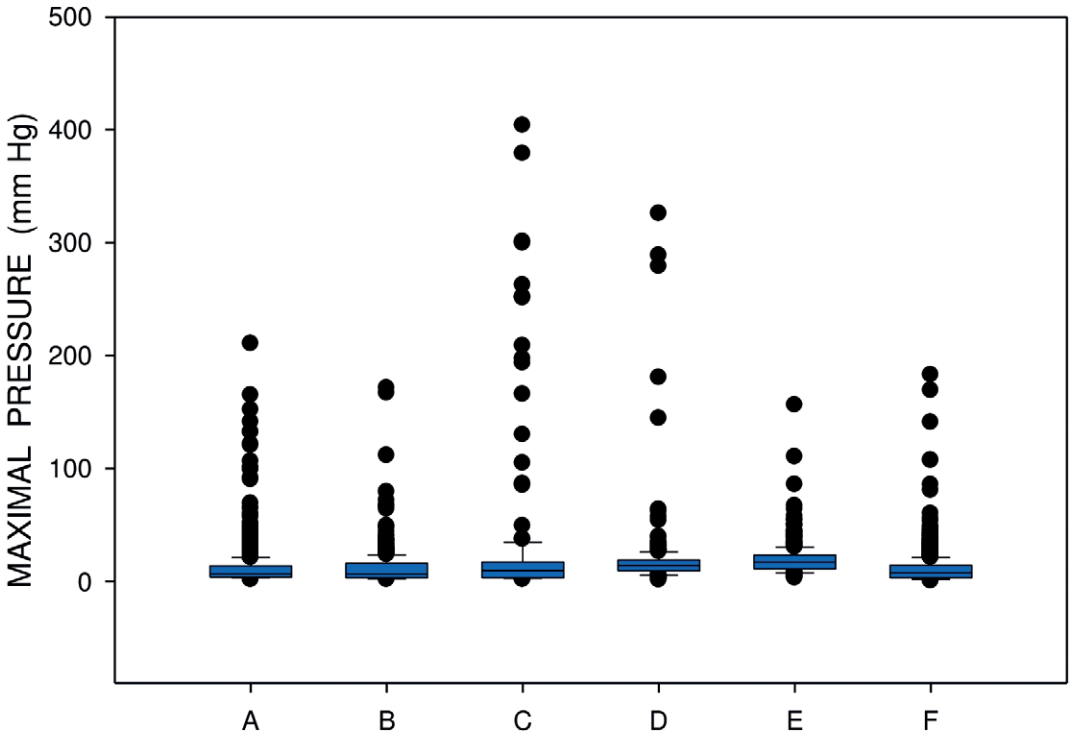

Fig. 6 Maximal intra-luminal pressure. Group A: stomach under fasting condition; B: stomach after food intake; C: passage of a capsule from the stomach to the duodenum; D: jejunum; E: ileum; F: colon. Median, inter-quartile range and outliers. Selected statistically significant differences: A > B ($p = 0.018$); C > B ($p = 0.014$); E > D ($p < 0.001$); E > F ($p < 0.001$).

wireless motility capsules in experimental pigs was published by Henze et al. (32). They investigated four male piglets (15–17 kg). Gastric emptying time under fasting conditions ranged from 68 to 233 hours. Transit times through the porcine small intestine were much more consistent than for the gastric compartment and ranged between 2–4 hours. The mean colonic transit time in this study was highly variable (21–169 hours) (32).

Warrit et al. (41) studied wireless motility capsules in adult healthy dogs. Median gastric emptying time was 20 hours (wide range 6–119 hours). Gastric pressure pattern and pH was dependent on the phase of food consumption. Mean small intestinal transit time was 3 hours (range 2–5 hours). Mean large bowel transit time was 21 hours (range 1–69 hours). There was a considerable inter-individual variation in motility patterns and transit times in dogs (41).

Last but not least, it is necessary to point out that both ketamine-based induction of general anaesthesia as well as long-lasting general anaesthesia (> 6 hours) were associated with a retention of motility capsules in the porcine stomach during the entire 5-day period. In our previous study on electrogastrography in experimental pigs we found out that ketamine, administered even in a single intramuscular dose, affected myoelectric function of the porcine stomach (42). Henze et al. (32) also found a capsule retention in the stomach in 3 of 8 piglets in their study. The authors did not mention what type of anaesthesia they used and did not state if ketamine was omitted for the induction of general anaesthesia (32).

Clinical use of wireless motility capsules has been reported since early 2010s (43–46). Motility pattern was studied in gastroparesis (43), irritable bowel syndrome (44) and for assessment of the effect of different drugs (e.g. erythromycin or morphine) (45). Wireless motility data correlated with scintigraphy in delayed gastric emptying (46).

Wireless, non-invasive complex investigation of gastrointestinal function will enable future experimental studies of gastrointestinal side effects of oncology chemotherapy. It also will facilitate further research of acetylcholinesterase inhibitors, modulators and re-activators and last but not least will extend possibilities of preclinical pharmacokinetic projects.

We are aware of possible limits of this pilot study. In spite of the assisting dedicated software that suggests time frames, we were not able to set time intervals fully precisely. Different parameters were combined to estimate particular periods. Fasting condition is associated with low gastric pH while intake of food is characterized by an increase of pH and temperature. The passage of a capsule from the stomach to the duodenum is associated with an increase of intra-luminal pressure and pH. Intra-luminal pressure in the colon is lower compared to the small intestine. The most questionable point is the time border between the jejunum and ileum. It is impossible to set it exactly even at porcine gross anatomy and histology (with an assumption of two equal lengths) (47–51).

CONCLUSIONS

Our methodical study has proven that a long-term function monitoring of the gastrointestinal tract by means of

wireless motility capsules in experimental pigs is feasible. However, both ketamine-based induction of general anaesthesia as well as long-lasting general anaesthesia (> 6 hours) should be avoided to prevent retention of a capsule in the porcine stomach.

ACKNOWLEDGEMENTS

The authors are much grateful to Professor Brendan T Griffin, School of Pharmacy, University College Cork, Ireland, for his highly-valued advice and recommendations.

The authors thank Richard Uhlíř, Daniel Kolář, Lenka Lacková and Jana Ďurišová for their excellent technical cooperation and support.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

FUNDING

This work was supported by research projects DZVRO MO1012 and MH CZ – DRO (UHHK, 00179906).

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Antithrombin Deficiency: Frequency in Patients with Thrombosis and Thrombophilic Families

Maria Anna Pejková[1], Eva Ivanová[1], Petr Sadílek[1], Radovan Malý[2], Zuzana Thibaud[1], Petr Dulíček[1,*]

ABSTRACT

Purpose: Antithrombin (AT) deficiency is a well-known inherited risk factor for venous thromboembolism (VTE). However, F V Leiden and F II20210a mutations have drawn much more attention in the recent years. Therefore, we have decided to analyze the frequency of antithrombin deficiency in different cohorts of patients and tried to formulate indications for its testing.

Results: Antithrombin deficiency was found in 4% of patients with recurrent VTE ≤ 50 years of age with, in 1% of patients with splanchnic vein thrombosis and in 2% of cases associated with combined oral contraceptives (COC) use or pregnancy. In patients with central venous thrombosis, antithrombin deficiency was not found.

Recommendation: We consider antithrombin testing useful in patients with thrombosis occuring up to 45 years of age without any risk factors. Namely, females with VTE in pregnancy and puerperium should be tested as well as females with thrombosis on COC, if VTE occurred within the first year of their use.

Conclusion: In spite of degressive interest in thrombophilia work up, we still consider antithrombin testing useful in defined clinical situations.

KEYWORDS

thrombophilia; venous thromboembolism; antithrombin; pregnancy

AUTHOR AFFILIATIONS

[1] 4th Department of Internal Medicine – Hematology, Faculty Hospital, Charles University, Faculty of Medicine in Hradec Králové, Hradec Králové; Czech Republic

[2] 1th Department of Internal Medicine – Cardiology, Faculty Hospital, Faculty of Medicine in Hradec Králové, Charles University, Hradec Králové; Czech Republic

[3] 4th Department of Internal Medicine – Hematology, Faculty Hospital, Charles University, Faculty of Medicine in Hradec Králové, Hradec Králové; Czech Republic

* Corresponing author: 4th Department of Internal Medicine – Hematology, Faculty Hospital, Charles University, Faculty of Medicine in Hradec Králové, Hradec Králové; Czech Republic; e-mail: dulicpet@fnhk.cz

Received: 7 January 2023
Accepted: 10 May 2023
Published online: 26 June 2023

Acta Medica (Hradec Králové) 2023; 66(1): 19–23
https://doi.org/10.14712/18059694.2023.10

INTRODUCTION

Antithrombin (AT) is a natural anticoagulant that plays a pivotal role in coagulation homeostasis. The target proteases of AT are those of contact activation pathway (formerly known as the intrinsic pathway), namely the activated forms of Factor X (Xa), Factor IX (IXa), Factor XI (XIa), Factor XII (XIIa) and, to a greater extent, Factor II (thrombin) (IIa) (1). Factor VII (VIIa) from the tissue factor pathway and kallikrein are inactivated too.

Normal plasma activity levels are in the range of 80–120%, where 100% of AT corresponds to 1 unit of antithrombin in 1 mL of reference plasma. With congenital AT deficiency, functional AT levels are often reduced to 40–60% of normal. AT deficiency as an inherited risk factor for venous thromboembolism (VTE) was first described by Olav Egeberg in 1965 (2). The interest in inherited thrombophilia has dramatically accelerated after the discovery of APC resistence (3) and detection of F V Leiden (4) and F II202010a mutations (5). Over the next decade, thrombophilia work-up was widely available and excessively used in daily routine work. However, since the precise knowledge of its clinical implications became more clarified, the interest in testing somewhat fainted away and thrombophilia testing, including testing for AT deficiency, has undergone a critical reappraisal (6). Nevertheless, thrombophilia work-up should be done on individual basis with the focus on further benefit for certain group of patients (7). AT deficiency is a strong risk factor for venous thromboembolism, particularly its increased recurrence rate compared with patients with mild thrombophilia (8). The absolute risk of the first and recurrent VTE was summarized (9). Based on 19 studies, odds ration (OR) estimates for the first VTE is 14.0 (95% credible interval (CrI), 5.5 to 29.0). Based on 10 studies, meta-analysis showed that the annual VTE risk was significantly higher in AT deficient 1.2% (95% CrI, 0.8–1.7) than in non-AT deficient individuals 0.07% (95% CrI, 0.01–0.14). In prospective studies, the annual VTE risk in antithrombin deficient individuals was as high as 2.3% (95% CrI, 0.2–6.5). The OR for recurrent VTE based on 10 studies was 2.1 (95% CrI, 0.2 to 4.0). The annual recurrence risk without long-term anticoagulant therapy based on 4 studies was 8.8% (95% CrI, 4.6–14.1) for antithrombin-deficient and 4.3% (95% CrI, 1.5–7.9) for non-AT deficient VTE patients [9]. The incidence of inherited AT deficiency has been estimated between 1 : 2000 and 1 : 3000 in the normal population (10), but precise data from Czech Republic are not available.

THE AIM OF THE STUDY

Our goals were:

1. Finding out the frequency of AT deficiency in several subgroups of patients with VTE.
2. Formulation of the indications for AT testing in patients with VTE in our Centre.

Why have we decided to analyze AT deficiency?

1. It is assocciated with increased risk of recurrence of VTE.
2. Therapy can have some impact in specific clinical situations:
   a) AT concentrate use in high risk situations or in therapy of acute thrombosis,
   b) heparin therapy is not adequate in some subtypes of AT deficiency.
3. It is important for females in the management of subsequent pregnancies and deliveries.

MATERIALS AND METHODS

We analyzed frequency of AT deficiency in the following cohorts of patients:

1. with VTE – deep vein thrombosis (DVT) and pulmonary embolism (PE) according to our criteria until 2002 or with a strong positive family history of VTE (11),
2. with splanchnic vein thrombosis (SVT),
3. with thrombosis in central nervous system, i.e. central venous thrombosis (CVT), stroke and transitory ischemic attack (TIA),
4. with thrombosis in all areas in association with pregnancy or COC use.

Patients were recruited within the last 20 years (1998–2018) in our thrombosis Center.

TESTING OF ANTITHROMBIN

Blood samples were collected by venipuncture into plastic tubes containing 1/10 volume of 3.8% sodium citrate for coagulation assays. AT level was determined by chromogenic assay using the Stachrom AT kit (STAGO D; normal value 80–120%). The normal range for AT was obtained by examination of 100 healthy individuals (50 men, 50 women) from our region, and normal values were compared with the normal ranges recommended by the manufacturer.

The presence of AT deficiency was accepted only after multiple testing with elimination of bias. Decreased activity was found in acute thrombosis, on heparin therapy and was also slightly decreased in COCs users and pregnancy. On the contrary, level can be somewhat increased on coumadine therapy. DOACs also have an impact on measurements of AT (12). We did not have to measure antigen levels as it is recommended by Scientific Subcommittee of the International Society on Thrombosis and Haemostasis (13).

We have also tested other thrombophilias: F V Leiden and F II20210a mutations, protein S and C deficiencies. Antiphosholipid antibodies and lupus anticoagulant were tested as well.

RESULTS

1. PATIENTS WITH VTE – DEEP VEIN THROMBOSIS (DVT) AND PULMONARY EMBOLISM (PE)

Since 1998 till 2002 we performed complete thrombophilia work-up in 325 patients (the mean age of 1st VTE was 33.5 y.). Every patient had to fulfill at least one of the following criteria:

1. VTE ≤ 50 years of age.
2. Recurrent VTE.
3. VTE at an unusual site.

4. Patients with recurrent thrombophlebitis (without varicose veins) (≥ 3 events).
5. Individuals with a positive family history of VTE ≤ 50 y. of age.

The results are shown in table 1.

AT deficiency was found in 4% of patients. Females with VTE in association with COCs or pregnancy were calculated separetely (cohort 4).

Tab. 1 AT deficiency and other thrombophilias in VTE.

Thrombophilia	%
F V Leiden	40.3
F II20210a	5.8
AT deficiency	**4.0**
Proteinu C def.	6.2
Proteinu S def.	10.5
APS /LA, ACa/	6.0

2. PATIENTS WITH SPLANCHNIC THROMBOSIS

We have assessed AT deficiency in a cohort of 90 patients with thrombosis of portal, mesenteric, splenic veins and Budd-Chiari syndrome. The patients were recruited between 2012 and 2019 with the first event ≤ 50 years. Patients with tumor or liver cirrhosis were not included.

Results are summarized in table 2.

AT deficiency was found in 1%, in one male with spontaneous portal vein thrombosis.

Tab. 2 AT deficiency in SVT + other thrombophilia.

SVT	90	Male 35	Female 55
Mean age (range)	38 (16–76)	41 (19–52)	36 (16–76)
F V Leiden	10	5	5
F II20210a	5	3	2
Deficit PC	5	2	3
APS	5	3	2
JAK-2 kinase	25	10	15
AT deficiency	1	1	0
Deficit PS	0	0	0

3. PATIENTS WITH THROMBOSIS IN CENTRAL NERVOUS SYSTEM

Antitrombin deficiency was evaluated in group of 50 patients with thrombosis in CNS.

Results are shown in table 3.

AT, PC, PS deficiency were not detected in this cohort.

4. FEMALES WITH VTE AND CNS THROMBOSIS IN ALL LOCALISATIONS AND IN ASSOCIATION WITH PREGNANCY OR COC USE

The onset of all cases of AT deficiency during COCs use was during the first six months of use. Proximal DVT was found in all cases, 10 cases represented pulmonary embolism. DVT in pregnancy was manifested in the third trimester and it was always proximal or pelvic.

Tab. 3 AT deficiency in CVT + other thrombophilia.

CVT	50	Male 22	Female 28
Mean age	36 (17–78)	40 (17–73)	35 (17–78)
F V Leiden	5	3	2
F II20210	5	2	3
APS (LA, ACa)	4	2	2
JAK -2 kinasa	2	1	1
AT deficiency	0	0	0
Deficit PC	0	0	0
Deficit PS	0	0	0

Tab. 4 AT deficiency on COCs.

Number (N)	850	
Mean age (years) at the time of thrombophilia work-up (range)	32 (16–50)	
Mean age (years) at the time of the first VTE (range)	26 (16–50)	
Antitrombin deficiency (N, %)	17 (2)	
COCs + VTE (DVT + PE) (N) COCs + stroke, TIA, CVT Pregnancy	730 70 50	15 p. = 2.0% 1 p. = 1.4% 1 p. = 2.0%

If we summarize the entire cohort of 1315 patients with various forms of thrombosis, with the first thrombotic event ≤ 50 years of age in 95.5% (N = 1255 p.), AT deficiency was found in 2.3%.

DISCUSSION

Thrombophilia testing has been widely accessible in the new millenium. However, in most of the cases of thrombophilia testing, results have had no clinical consequences. Despite these findings, thrombophilia work-up is still done more frequently than necessary in the Czech Republic, mainly F V Leiden and F II20210a mutations. The awareness about other thrombophilias has fainted. Congenital AT deficiency is an infrequently encountered genetic risk factor for VTE and different subtypes vary with regard to their thrombotic risk (14). Therefore, we decided to analyze frequency of AT deficiency in different cohorts of patients and formulate the indication for AT testing.

Concerning CVT, we have not found significant AT deficiency, except in 1.4% subset of females in the 4th cohort. In the large international study CEVETIS, AT deficency was revealed in 2% (564 patients were tested) (15). In other similar studies, frequency of AT deficiency was around 2% (4/172 patients) as well. (16).

Frequency of AT deficiency was found in 1% in our cohort with splachnic thrombosis, which was lower in comparison with 4.5% found in a similar size of cohort (17).

In our first cohort of patients with complete thrombophilia-work up, AT deficiency was found in 4%, therefore more frequently than in other studies, where among 1165 of individuals with unprovoked VTE, AT deficiency was

detected only in 1% (18). However, we used different criteria for testing, mainly in the term of age limit.

In females of reproductive age, AT deficiency was found in 2% in association with COCs and 2% with pregnancy. In cases on COCs, the onset of VTE was in 90% of cases during the first 6 months of pill introduction and in 100% within one year. Regarding the severity of the event, thrombosis was always clinically significant. It means only proximal DVT, PE or CVT were diagnosed. According to a meta-analysis of 12 case–control and three cohort studies, severe thrombophilia increased the risk of VTE on COCs 7-fold (RR, 7.15; 95% CI, 2.93–17.45) (19). Seven case–control studies showed the incidence of antithrombin, protein C and protein S deficiency in COC-users in 4.3 (95% CI, 1.4– 9.7) to 4.62 (95% CI, 2.5–7.9) vs. 0.48 (95% CI, 0.1–1.4) to 0.7 (95% CI, 0.0–3.7) per 100 pill-years in non-deficient COC-users (20,21).

Regarding absolute risks of pregnancy associated VTE, high risk was found in AT deficiency (antepartum: 7.3%, 95% credible interval 1.8% to 15.6%; post partum: 11.1%, 3.7% to 21.0% (22). We found that diagnosis of AT deficiency is important, especially in reproductive age, because the risk of pregnancy related VTE and its obstetrical complications is significant (23–26).

Based on our results we have formulated the strategy of AT evaluation. We consider testing of AT useful in all males with unprovoked VTE up to 45 years of age, as well as for all females at this age category, who were not pregnant or did not take COC. Females with VTE in pregnancy and puerperium are tested as females with VTE in association with COC, when VTE is at least proximal and within the first year of use. We recommend long-term anticoagulation after idiopathic VTE and thromboprophylaxis in subsequent pregnancies. However, final management decisions in such cases ultimately hinge on individualized consideration of the benefits and risks of anticoagulation along with patient preference rather than on an algorithmic pathway (7).

We are avare of some shortcomings. There are several subtypes of congenital AT deficiency with different thrombophilic potential. We did not measure the antigen so that we cannot subclassify our patients. Gene mutation testing is available only in limited number of patients.

CONCLUSION

It is the 58th anniversary of the first description of AT deficiency. In the last 25 years the strategy for thrombophilia work up has changed and nowadays it is less recommended. We recommend thrombophilia work-up of idiopathic thrombosis based upon an individual assessment of each clinical scenario with particular emphasis on potential sequals for each patient separately. That is why we consider antithrombin testing useful in defined clinical situations.

ACKNOWLEDGEMENTS

MA Pejkova confirms that there are no conflicts of interest associated with this publication

THE WORK WAS SUPPORTED

This work was supported by the programme Cooperatio, Science in ONKO.

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The Sex Ratio at Birth Is Higher in Māori than in Non-Māori Populations in Aotearoa New Zealand

Victor Grech[1,*]

ABSTRACT

Aims: The sex ratio at birth approximates 0.515 (male : total, M/T), with 515 boys per 485 girls. Many factors have been shown to influence M/T including acute and chronic stress. Increasing maternal age is associated with a decline in M/T. In Aotearoa New Zealand, circa 15% of the population identify as of Māori heritage. This populationis generally considered to be socioeconomically disadvantaged. This study analysed M/T for Māori and non-Māori M/T births in Aotearoa New Zealand and relates these to mean maternal age at delivery.
Methods: Live births by sex and maternal age at delivery were available from the website of Tatauranga Aotearoa Stats NZ for 1997–2021.
Results: This study analysed 1,474,905 births (28.4% Māori) Pooled data shows that Māori M/T is significantly higher than non-Māori M/T (chi = 6.8, p = 0.009). Mean maternal age at delivery was less for Māori mothers but this was not statistically significant.
Conclusions: Several studies have shown that M/T is decreased in socioeconomically deprived populations, and for this reason Māori M/T is expected to be lower and not higher than non-Māori M/T. A lower mean maternal age at delivery might have explained the M/T differences noted in this analysis but this was not a statistically significant difference.

KEYWORDS

humans; sex ratio; New Zealand; age; maternal; native Hawaiian or other Pacific Islander

AUTHOR AFFILIATIONS

[1] Consultant Paediatrician, University of Malta, Malta
* Corresponding author: Paediatric Dept, Mater Dei Hospital, Malta; e-mail: victor.e.grech@gov.mt

Received: 17 July 2022
Accepted: 20 January 2023
Published online: 26 June 2023

Acta Medica (Hradec Králové) 2023; 66(1): 24–27
https://doi.org/10.14712/18059694.2023.11

INTRODUCTION

The sex ratio at birth is expected to approximate 0.515 (male to total births, M/T), such that 515 boys are born for every 485 girls (1). A wide range of factors have been shown to influence M/T (2), including acute stressful events and these may be not only natural events, such as earthquakes and floods (3, 4), but also man-made such as terrorist attacks and contracting economies (5, 6). Races have been shown to have different M/T ratios and it has been speculated that these may be innate (7), or due to environmental factors such as chronic stress (8). Increasing maternal age is associated with a decline in M/T, a factor that should be accounted for when comparing large groups, if possible (9).

In Aotearoa New Zealand, circa 15% of the population identify as of Māori heritage, tangata whenua (Indigenous people) and 7.8% of Pacific heritage (10). The Māori are a minority population originating from Australasia and compared other local populations, are overall considered to be socioeconomically disadvantaged (11, 12) and experience the poorest health statistics like many Indigenous peoples around the world (13–15).

This study was carried out to ascertain whether there were any significant M/T differences between Māori and non-Māori M/T ratios in Aotearoa New Zealand and to relate this to mean maternal age at delivery.

METHODS

Live births by sex were available as quarterly data from the website of Aotearoa New Zealand's national statistics office, Tatauranga Aotearoa Stats NZ (16). Data for Māori and total births (from which non-Māori were obtained by subtraction) were available for contiguous years for the period 1997–2021. 95% confidence intervals for M/T were calculated using the equations of Fleiss (binomial) (17). Mean maternal age at delivery was available for December for each year for the period studied for Māori and non-Māori births.

Data was analysed in bespoke Excel sheets as created for the Write a Scientific Paper Course (WASP), including

Tab. 1 Māori and non-Māori male and female births and M/T with 95% confidence intervals.

1997–2021	Māori	Non-Māori
M	215,790	540,702
F	203,538	514,875
Total	419,328	1,055,577
UCI	0.5161	0.5132
M/F	0.5146	0.5122
LCI	0.5131	0.5113

Tab. 2 Maternal ages at birth (1997–2021), Māori and non-Māori births.

	Māori	Non-Māori
Weighted mean	26.37	30.15
Weighted median	26.00	30.00
Weighted SD	7.03	10.25
Conf. Int. (95%)	2.49	3.63

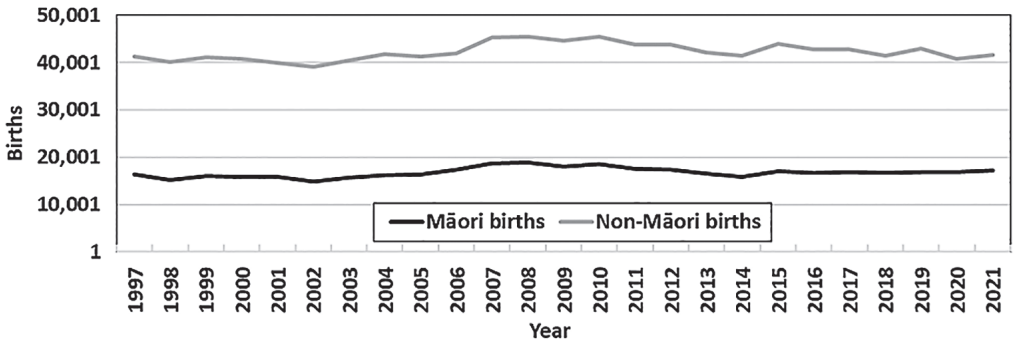

Fig. 1 Live births, Māori and non-Māori, 1997–2021.

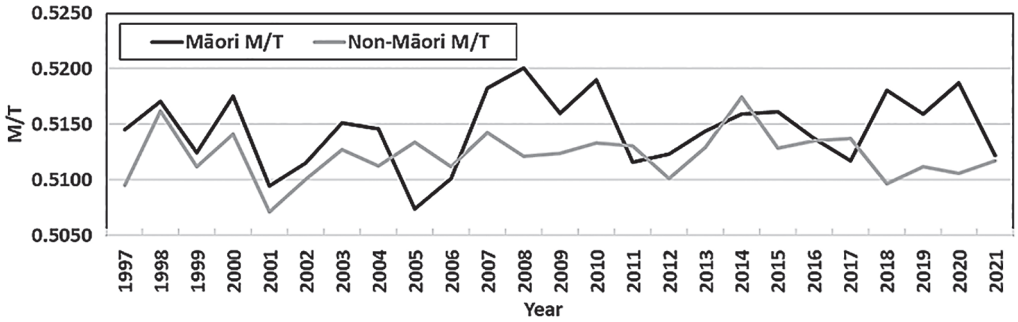

Fig. 2 M/T, Māori and non-Māori births, 1997–2021.

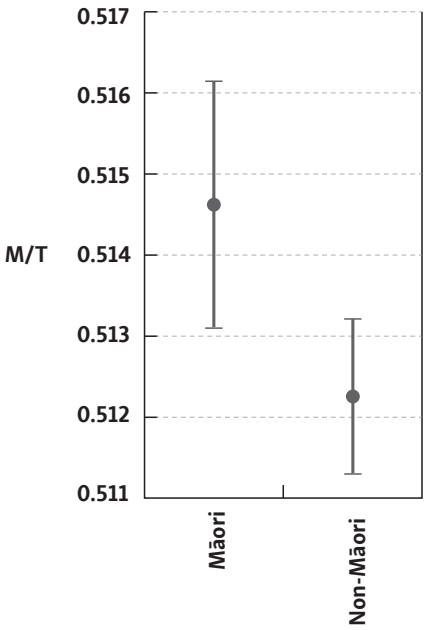

Fig. 3 Pooled M/T (1997–2021), Māori and non-Māori births.

chi tests, confidence intervals for proportions and weighted means t tests (18). A p value of ≤ 0.05 was taken to represent a statistically significant result.

RESULTS

This study analysed 1,474,905 births (28.4% Māori) over the period 1997–2021. Birth rates were stable (Figure 1) and M/T variations were within expected 95% confidence intervals with Māori M/T overall generally higher than non-Māori M/T (Figure 2). Pooled data in table 1 shows that Māori M/T is significantly higher than non-Māori M/T for the study period (chi = 6.8, p = 0.009 – Figure 3).

Maternal ages at delivery are plotted in figure 4. The distributions appear different with younger Māori mothers overall, and summary statistics are shown in table 2. However, t-testing for weighted means showed no significant difference between weighted mean maternal ages at delivery for Māori and non-Māori births (t = 1.74, p = 0.086 (two-tailed).

DISCUSSION

Internal population M/T differences have been described. For example, Hungarian Gypsies invest more heavily in daughters than sons when compared with the rest of the Hungarian population (19). Similarly, differences have been noted in a more modern U.S. populations and in and Kenyan Mukogodo hunter-gatherers (20, 21).

Racial M/T differences had been historically ascribed to innate racial differences (7). However, it has been noted that in the United States that M/T is Asian or Pacific Islander > White > American Indian or Alaska Native > Black or African American. The lower M/T of Indian or Alaska Native and Black and African American in this country is tantamount to a constant loss of 3.5–4/1000 male births when compared to White M/T. Since race is the most significant variable associated with wealth inequality in the US, chronic socioeconomic stress may be responsible, in part or in full, for this M/T difference (8, 22). Similarly, in a large, United Nations dataset, it has been shown that M/T was More Developed Countries > Least Developed Countries (23), supporting the socioeconomic hypothesis for lower M/T due to chronic stress (24). This is in accordance with the Trivers-Willard hypothesis of parental investment which avers that natural selection has favoured parents who adapt to periconceptual and early pregnancy conditions, such as in conditions of stress, weaker (typically male) foetuses are lost paving the way for a new pregnancy that may be female and likelier to survive to term and to adult life and become pregnant, or another son perhaps under better conditions (25). Poor socioeconomic conditions are tantamount to chronic stress and may result in chronic lower M/T ratios (24).

Overall, M/T in Aotearoa New Zealand has been shown to be stable over the last few decades (26). The minority Māori are generally considered to be socioeconomically disadvantaged (11–15), and based on the socioeconomic hypothesis of M/T, Māori M/T is expected to be lower and not higher than non-Māori M/T. However, other factors may have influenced/biased M/T in these populations.

Increasing maternal age has been shown to be associated with a decline in M/T in offspring (9, 27, 28). It has been speculated a higher maternal age may in and of itself be a stressful factor in pregnancy (29), and since there is evidence that female live births are overrepresented in

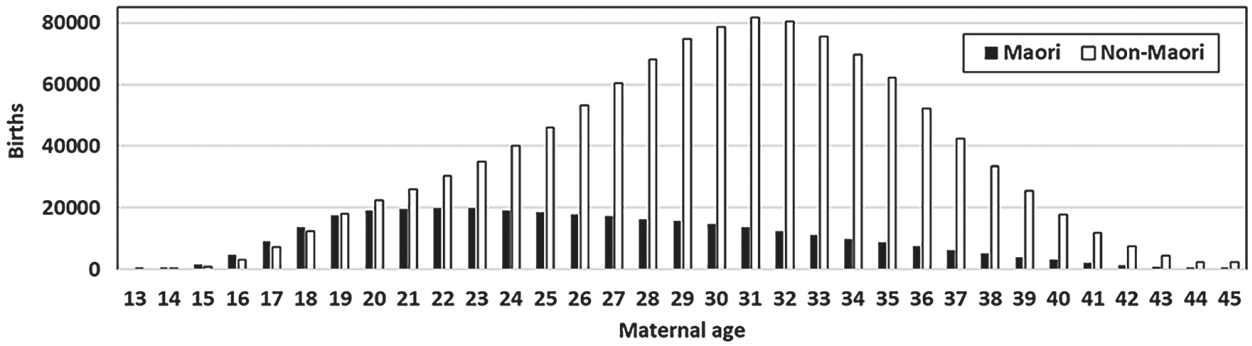

Fig. 4 Maternal ages at birth (1997–2021), Māori and non-Māori births.

stressful pregnancies (30), a lower mean maternal age at delivery might have explained the M/T differences noted in this analysis.

This study is limited by several factors. In the first instance, the lack of a statistically significant difference in mean maternal age between Māori and non-Māori may have been due to lack of power, with inadequate numbers. Furthermore, many factors influence M/T (2), including toxins (31), and the datasets available were very limited in scope and breadth, without individual maternal data.

Finally, all such studies are hampered by publicly available data that is insufficiently detailed, for example, monthly births by sex, despite the obvious fact that this data is routinely collected. National statistics offices may view the Centers for Disease Control natality database (CDC Wonder) as a suitable example for data availability, allowing researchers access to anonymous data for detailed studies (32). In practice, only very rarely do researcher gain access to highly detailed, anonymised data by individual, and this is typically data obtained from a researchers' own countries (33). In conclusion, M/T remains a fascinating field of study and access to more detailed data would immensely help researchers.

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Drug Rash with Eosinophilia and Systemic Symptoms (DRESS Syndrome)

Marta Filipa Lemos Mendes[1,*], Diana Silva Fernandes[1], Ilídio Brandão[1]

ABSTRACT
DRESS syndrome is an idiosyncratic drug reaction and potentially life-threatening. The authors report a case of this syndrome presenting with fever, rash, mucosal involvement, liver and muscle involvement associated with moxifloxacin treatment.

KEYWORDS
DRESS; drug adverse reactions; moxifloxacin; hypersensitivit; eosinophilia

AUTHOR AFFILIATIONS
[1] Department of Internal Medicine, Hospital de Braga – Sete Fontes – São Victor 4710-243 Braga, Portugal
* Corresponding author: Department of Internal Medicine, Hospital de Braga, Serviço Medicina Interna, Hospital de Braga, Sete Fontes – São Victor 4710-243 Braga, Portugal; e-mail: mmartaflmendes@gmail.com

Received: 9 October 2022
Accepted: 15 February 2023
Published online: 26 June 2023

Acta Medica (Hradec Králové) 2023; 66(1): 28–31
https://doi.org/10.14712/18059694.2023.12

INTRODUCTION

Drug-induced hypersensitivity syndrome (DRESS) is a drug-induced hypersensitivity reaction that is rare (the estimated incidence is between 1 in 1000 and 1 in 10,000 drug exposures) and potentially life-threatening, often involving skin rashes, hematologic abnormalities (eosinophilia, atypical lymphocytosis), lymphadenopathy, and internal organ involvement. The pathogenesis is not fully understood. Clinical manifestations often occur 2 to 8 weeks after initiation of treatment with the causative drug, although a new reaction may occur within hours to days (1, 2).

Diagnostic criteria for DRESS syndrome published in 1996 by Bocquet et al., include the simultaneous presence of three conditions drug-induced rash, eosinophilia ≥ 1500/mm^3, and at least one of the following systemic abnormalities: lymphadenopathy, hepatitis (transaminases > 2 ULN), interstitial nephropathy, interstitial lung disease, myocardial involvement.

CASE DESCRIPTION

An 83-year-old man was hospitalized with a febrile and pruritic morbilliform rash that rapidly developed into erythroderma (Figs. 1–4). A week earlier, he had seen a physician for acute tracheobronchitis and received a prescription for moxifloxacin. Physical examination revealed coalescing erythematous macules and papules, on the upper trunk, face, and extremities, fever of 38.5 °C and whitish, painful mouth ulcers. There was no evidence of lymphadenopathy or hepatosplenomegaly. Standard laboratory tests showed leukocytosis with eosinophilia (15100 leukocytes/μL and 1223 eosinophils/μL) without atypical lymphocytes, alanine transaminase 80 U/L [7–40] and aspartate transaminase 60 U/L [12–40], creatine kinase 302 U/L [46–171] and myoglobin 764 ng/mL [<110], troponin I 0.017 ng/mL [< 0.045], creatinine 0.9 mg/dL [0.7–1.20] and urea 44 mg/dL [19–49]. A CT chest, abdomen and pelvis was performed, which revealed bilateral pleural reaction, with no evidence of lymphadenopathy. Other causes were excluded, namely negative antinuclear antibodies, negative blood cultures, and negative serology for EBV, CMV, herpesviruses, HAV, HBV, HCV, *chlamydia*, and *mycoplasma*. Moxifloxacin was discontinued and the patient was treated with prednisolone 1 mg/kg/day daily, with gradual resolution of lesions and improvement in analysis.

DISCUSSION

Given clinical complexity, heterogeneity in presentation, and overlapping features with other diseases, various scoring systems and guidelines have been suggested over the

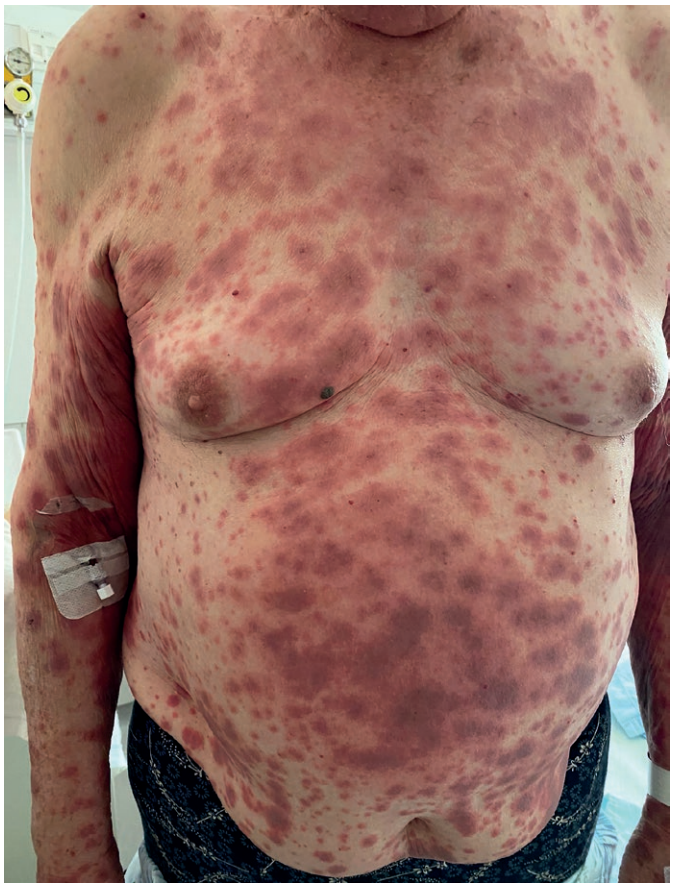

Fig. 1 Clinical presentation of DRESS syndrome: rash with confluent plaques and purpura.

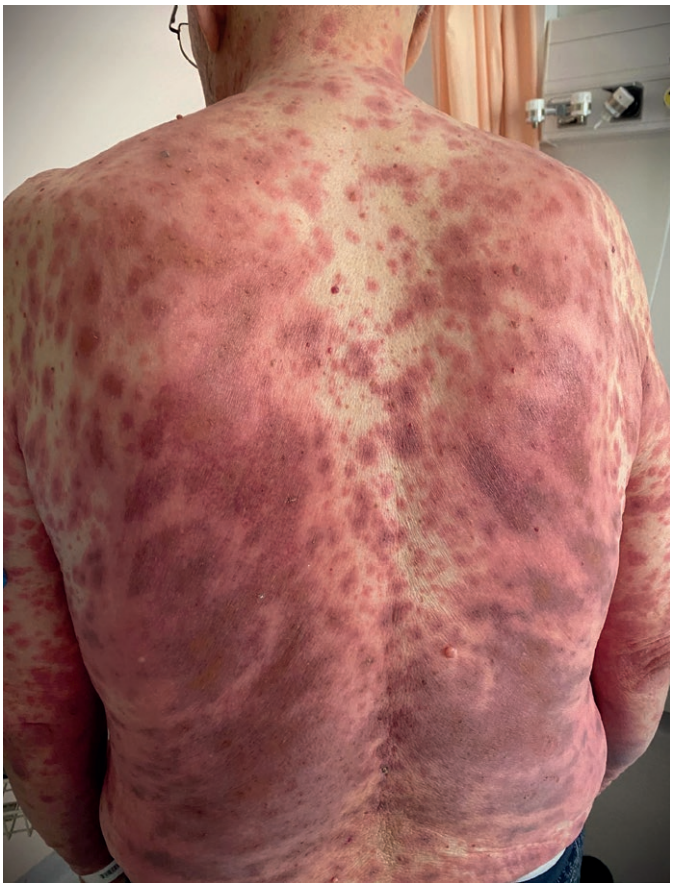

Fig. 2 Clinical presentation of DRESS syndrome: rash with confluent plaques and purpura.

Fig. 3 Clinical presentation of DRESS syndrome: rash with confluent plaques and purpura.

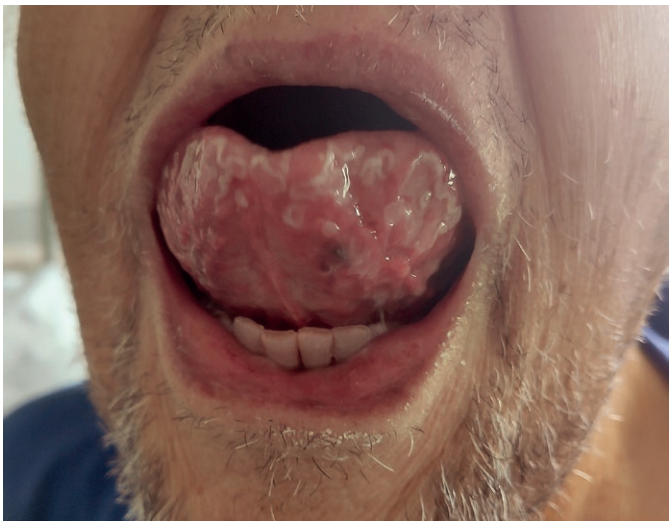

Fig. 4 Clinical presentation of DRESS syndrome: mucosal involvement.

last 25 years to facilitate the diagnosis of DRESS. The recently published Spanish guidelines for DRESS advise the use of RegiSCAR criteria in clinical diagnosis (3). Thus, in this patient, the diagnosis of DRESS syndrome was based on the presence of febrile rash, mucosal involvement, eosinophilia, liver and muscle involvement in a patient who had started therapy with moxifloxacin one week before (RediSCAR 6 – Table 1).

The diagnosis of DRESS syndrome implies a high level of suspicion. It is associated with prolonged hospitalization

Tab. 1 RegiSCAR Validation Score for DRESS Syndrome 2007.

Score	-1	0	1	2
Fever≥38.5 (core) or >38°C (axilarry)	No	Yes		
Enlarged lymph nodes (>1 cm size, at least 2 sites)		No/Unknown	Yes	
Eosinophilia		No/Unknown	700–1499/μL 10–19.9% (if leukopenia)	³1500/μL ³20% (if leukopenia)
Atypical lymphocytes		No/Unknown	Yes	
Skin involvement			max 2 points	
Rash extent (%BSA)		No/Unknown	>50%	
Rash suggesting DRESS (≥2 of facial edema, purpura, infiltration, desquamation)	No	Unknown	Yes	
Biopsy suggesting DRESS	No	Yes/Unknown		
Organ involvement		No/Unknown	max 2 points	
Liver			Yes	
Kidney			Yes	
Lung			Yes	
Muscle/Heart			Yes	
Pancreas			Yes	
Other			Yes	
Resolution >15 days	No	Yes		
Evaluation of other potential causes			Yes (None [+] and at least 3 [–])	
Serology for HAV, HBV, HCV; Blood culture				
Antinuclearantibody; Clamydia/Mycoplasma				
Total score: <2, Excluded; 2–3, Possible; 45, Probable; >5, Definite				

and significant mortality risk, around 10%, mainly due to liver failure. Here we report a typical presentation to a not so typical medicine. Drug prompt withdrawal and organ support is essential. Better understanding of the syndrome pathogenesis shall allow us to standardize treatment, as it still remains empirical and with no established regimens.

In the case presented, a good evolution was observed.

ETHICAL CONSIDERATIONS

Declaration of interest: The authors received no support of any kind from public, private or nonprofit organisations.

Conflict of interest: The authors don't have conflict of interest.

Informed consent: Obtained.

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First Croatian Case of Double Aneuploidy: A Child With Klinefelter and Edwards Syndrome (48,XXY,+18) – Possible Causes and Contributing Factors

Ena Batinović[1,*], Anet Papazovska Cherepnalkovski[1,3], Bernarda Lozić[2,4], Luka Brajković[1], Ivana Zanchi[1], Vesna Pavlov[1], Marija Bucat[1]

ABSTRACT

We report a case of double aneuploidy in a preterm male newborn with karyotype 48,XXY,+18 whose mother was of advanced age and infected with the SARS-CoV-2 virus during the early stages of her pregnancy.

The clinical features observed in the newborn included intrauterine growth retardation, dysmorphic facial features, overlapping fingers on both hands, respiratory distress syndrome, ventricular septal defect, patent ductus arteriosus, persistent pulmonary hypertension, and bilateral clubfoot, a phenotype that mainly correlates with Edwards syndrome (trisomy 18).

To our knowledge, this is the first reported case of double aneuploidy in Croatia. This paper provides a detailed description of the clinical presentation and treatment strategies used, with the aim of providing valuable data for future recognition and management of similar cases. Furthermore, we discuss the mechanisms of nondisjunction that might account for this rare form of aneuploidy.

KEYWORDS

aneuploidy; COVID 19; genetic nondisjunctions; Edwards Syndrome; Klinefelter Syndrome

AUTHOR AFFILIATIONS

[1] Department of Neonatology, University Hospital Centre Split, Split, Croatia
[2] Department of Pediatrics, University Hospital Centre Split, Split, Croatia
[3] University of Split, University Department of Health Studies, Split, Croatia
[4] University of Split, School of Medicine, Split, Croatia
* Corresponding author: Department of Neonatology, Clinical Department of Gynecology and Obstetrics, University Hospital Centre Split; e-mail: ena.sardelic@gmail.com

Received: 2 February 2023
Accepted: 26 April 2023
Published online: 26 June 2023

Acta Medica (Hradec Králové) 2023; 66(1): 32–36
https://doi.org/10.14712/18059694.2023.13

INTRODUCTION

Double aneuploidy refers to the presence of two numerical chromosomal abnormalities in the same individual. The exact mechanism underlying the origin of double aneuploidy remains unclear. Some theories suggest that it may result from either two separate nondisjunctional events during gametogenesis or a single nondisjunctional event in a trisomic zygote (1). The simultaneous occurrence of two chromosomal aneuploidies in a living patient is extremely rare. The first documented case of a patient with double aneuploidy (i.e., 48,XXY,+21 karyotype), was described in 1959 (2). Since then, approximately 400 cases of double aneuploidy have been reported in the scientific literature (1, 3–6). It is probable that the lower frequency of double aneuploidies in the prenatal and postnatal period reflects a higher early intrauterine mortality rate of these fetuses, compared to fetuses with a single chromosome aneuploidy affecting either of the involved chromosome (7). The scarcity of literature data makes it challenging to accurately determine the prevalence of double aneuploidy in all recognized pregnancies.

Autosomal double trisomies are rarely reported in live-born infants and the reported cases of double aneuploidy mostly involve autosomal trisomy and sex chromosome trisomy (1, 8). Most double aneuploidies are associated with increased maternal age, an abnormal sonogram, and early pregnancy loss (1). However, children with sex chromosome aneuploidy and trisomies involving chromosomes 16, 18, and 21 may survive for longer gestation and even the postnatal period (9, 10). While there is limited research on the subject, COVID-19 during pregnancy could result in fetal complications, including intrauterine growth retardation, abortion, preterm delivery, or even stillbirth. (11)

The aim of this report is to describe clinical features and management strategies in a patient with double aneuploidy, to review and discuss mechanisms of nondisjunction as a probable underlying cause of aneuploidy, and to discuss the possible influence of COVID-19 parental infection on such chromosomal abnormalities.

CASE REPORT

The mother was 45 years old at the time of the child's birth. She previously had two uncomplicated pregnancies with normal outcomes and has two healthy children, both born at a gestational age of 41 weeks via spontaneous vaginal delivery.

The mother had a history of regular menstrual cycles, and her last menstrual period occured two weeks before she contracted COVID-19, at the very beginning of this pregnancy. She experienced relatively mild COVID-19 symptoms, which lasted for two weeks. Symptoms included fever that reached maximum value of 38 °C degrees and lasted up to five days, extreme fatigue, and upper respiratory tract symptoms. She was confirmed positive for SARS-CoV-2 through PCR testing. Her husband presented with similar symptoms at about the same time but was not tested for SARS-CoV-2. The mother also developed hypertension during the pregnancy which was treated with methyldopa. Ultrasound monitoring showed normal fetal anthropometric measurements and proper development up to the 17th pregnancy week. After that, the fetus showed signs of intrauterine growth retardation (IUGR) as well as polyhydramnios. Other fetal characteristics were normal. The mother denied alcohol or drug exposure during pregnancy. The infant was delivered via caesarean section at an estimated gestational age of 33 + 4 weeks due to non-reassuring fetal heart rate (NRFHR), IUGR, polyhydramnios, and pregnancy induced hypertension. The infant's birth weight was 1230 g (< 3rd percentile), length was 35 cm (< 3rd percentile), and head circumference was 27.5 cm (< 3rd percentile), respectively. APGAR scores were 4 after 1 minute and improved to 6 after 5 minutes. The infant was resuscitated using the T-piece resuscitator. After applying 5 initial sustained inflations lasting for 1 second each, a positive pressure ventilation was continued for 1 minute with a frequency of 40 ventilations per minute, peak inspiratory pressure (PIP) of 20 cmH_2O and FiO_2 rising from initial 21% to 30%. This resulted in an improvement of heart rate to 120 beats per minute and only a slight improvement of muscle tone. However, spontaneous breathing was inefficient and irregular with gasping, so the child was immediately intubated at the beginning of the second minute of life and was placed on mechanical ventilation for appropriate management of respiratory distress. Physical examination revealed several dysmorphic features, including a strawberry-shaped head, a depressed nasal bridge, microretrognathism, low set ears, widely spaced nipples, clenched fists with joint contractures, bilateral clubfoot, and a grade I–II pansystolic murmur. He also had bilaterally undescended testes with a normal size phallus and multiple body hematomas. A neurological exam revealed a hypotonus with normal reflexes. Laboratory findings showed metabolic acidosis, anemia, thrombocytopenia and elevated inflammatory markers, and diagnosis of early neonatal sepsis was made. He was placed, according to an established protocol, on a first-line double antibiotic regimen (β-lactam in combination with an aminoglycoside). A porcine surfactant preparation was administered intratracheally at a dose of 200 mg/kg within 30 minutes after delivery. A 4 F umbilical venous catheter was inserted and its depth was adjusted based on radiography findings. Adequate vascular volume and electrolyte balance were precisely maintained with IV fluids. Additional therapies included: caffeine citrate, calcium gluconate, and sedation with midazolam as well as vasopressor inotropic support. The chest X-ray showed bilateral lung infiltrates, while echocardiography revealed several cardiac abnormalities including the presence of a small muscular ventricular septal defect (VSD), patent foramen ovale (FOA), with a large patent ductus arteriosus (PDA) measuring 5 mm with a predominantly right to left shunt. In addition, the infant had, decreased left ventricular contractility, and supra-systemic pulmonary hypertension of 50 mmHg. An ultrasound of the brain showed no apparent abnormalities. Initially, the child was ventilated using synchronized intermittent positive pressure ventilation with volume guarantee (SIPPV + VG) with optimal adjustment of ventilatory parameters. On day 2, inhaled nitric oxide (iNO) at 20 ppm was introduced for

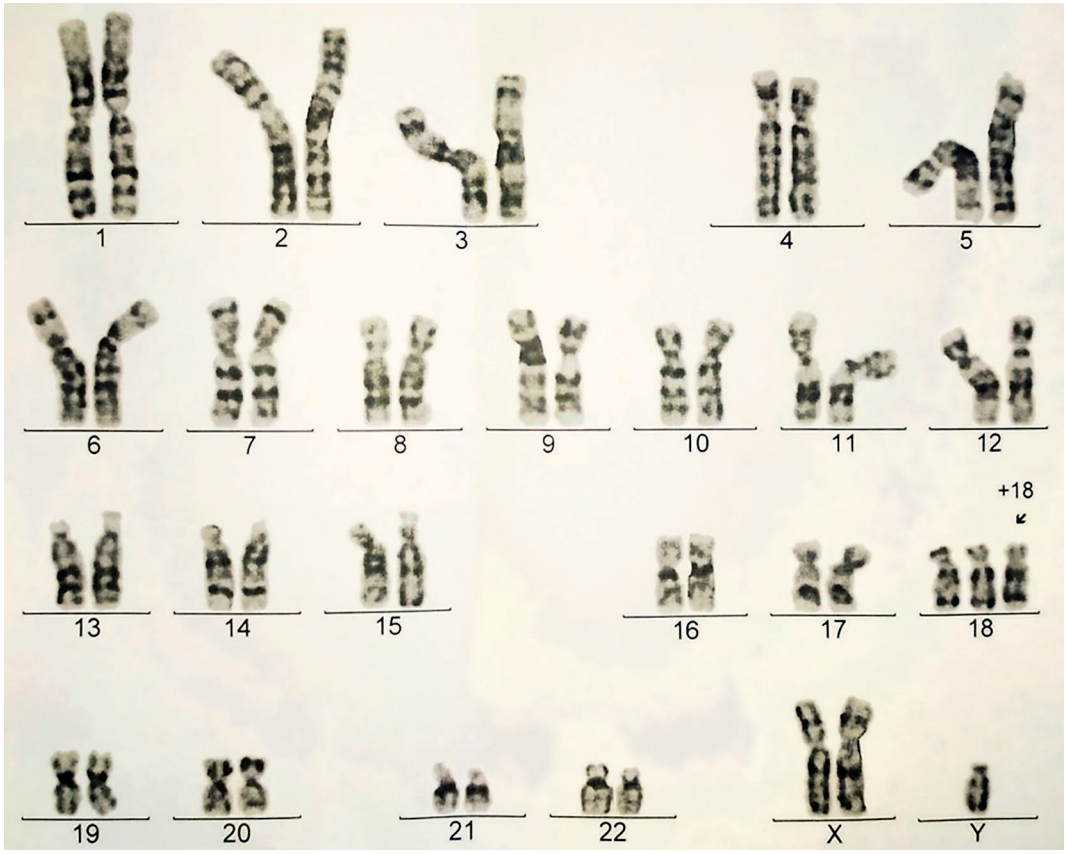

Fig. 1 Trypsin-Giemsa karyotype 48,XXY,+18 performed on cultured peripheral blood lymphocytes. Thirty metaphases were analyzed to exclude mosaicism and the double trisomy was seen in all analyzed metaphases.

the treatment of persistent pulmonary hypertension. Due to inadequate therapeutic response and increased oxygen requirements, the conventional ventilation was changed to high-frequency oscillatory ventilation (HFOV) with added inhaled nitric oxide (iNO). Despite receiving maximum respiratory support using conventional mechanical ventilation, the infant's condition continued to deteriorate, resulting in lethal outcome on fourth day of life. Chromosomal analysis of a peripheral blood sample, which came subsequently, using Giemsa trypsin (GTG banding) showed the karyotype of 48,XXY,+18 (Figure 1). The pathological (autopsy) finding reported a hypotrophic premature male child with the above mentioned dysmorphic and clinical features, bilateral pneumonia and pulmonary hyaloid membrane disease and normally placed internal organs, respectively.

DISCUSSION

The coexistence of Edwards and Klinefelter syndromes remains rarely reported in the literature. To the best of our knowledge, this double aneuploidy is the first reported case in Croatia.

Besides the common clinical characteristics in more prevalent Trisomy 18, it is very challenging to diagnose or suspect Klinefelter syndrome when these two conditions coexist.

Our child presented with typical facial features including a strawberry-shaped head, a depressed nasal bridge, microretrognathism, low set ears, widely spaced nipples, clenched fists with joint contractures, and bilateral clubfoot indicating Trisomy 18 but also had cryptorchidism which is commonly seen in both Trisomy 18 and Klinefelter syndrome (11, 12). In Trisomy 18 cardiac abnormalities are commonly seen but not so much in Klinefelter (11); however, in the combination of both trisomies, all the reported cases had cardiac involvement (PDA and VSD in more than 95% of cases) (12–14). Our patient had a grade I–II pansystolic murmur and was found to have VSD, FOA with a large PDA of 5 mm and pulmonary hypertension.

While the prenatal diagnosis remains extremely difficult, findings of polyhydramnios, IUGR, increased fetal nuchal translucency, or structural cardiac abnormality on prenatal ultrasound should cast doubt on the possibility of a chromosomal aberration (13). Consequently, while the coexistence of double aneuploidy is rare, the potential for combined abnormalities involving both autosome and sex chromosome should be raised even if clinical signs of one of the conditions are not present. Conventional karyotyping was performed on cultured peripheral blood lymphocytes to detect a possible chromosomal anomaly. Thirty metaphases were analyzed to exclude mosaicism and the double trisomy was seen in all analyzed metaphases before concluding the cytogenetic result. Cytogenetic analysis played the main role in establishing the diagnosis of our patient.

Meiosis is the basis of the reproduction process that ensures a reduction of ploidy and promotes genetic diversity in both males and females (15). Female germ cells

undergo the first meiotic division during embryonic development and arrest at the diplotene phase of prophase I before birth. After puberty with each menstrual cycle, some of the arrested oocytes within the preovulatory follicles may resume meiosis in response to luteinizing hormone (LH) surges. Soon after completing the first meiotic division, the second meiotic division starts and the oocyte arrests at metaphase II until fertilization. It is with fertilization only that the oocyte accomplishes its meiosis. Different factors including epigenetic molecules and different signaling pathways have been proven essential for proper meiotic maturation (16).

Regarding the mechanisms of nondisjunction, the parental origin of extra chromosomes and cell division level where nondisjunction occurred were not proved in our case. However, there is a clear association in literature between double aneuploidies and advanced maternal age, as was in our case. The extra chromosomes are mostly of maternal origin and the nondisjunction events can occur in different cell division stages (4, 17, 18). Furthermore, there is a clear association between advanced maternal age and chromosome laxity leading to nondisjunction. This is mainly due to inappropriate chromosome coiling and condensation in the oocyte of older women (19). A two-hit model of nondisjunction has also been suggested, in which the first hit is the prenatal formation of a susceptible tetrad, and the second hit is disruption of the meiotic process that increases the risk of nondisjunction of the susceptible configuration (20). Advanced maternal age remains the only well-documented risk factor for maternal meiotic nondisjunction, but there is, however, a surprising lack of understanding of the basic mechanisms behind maternal age. Recent association studies seem to support the compromised microcirculation hypothesis (21, 22).

As the COVID-19 infection broke out in 2019 it has rapidly turned into a global pandemic, becoming a healthcare burden, both for the health system and for patients. More and more questions are being asked about the female reproductive system, fertility problems and pregnancy outcomes, and an explanation is needed about the possible link between COVID-19 and women's reproductive health. Numerous studies so far have shown that SARS-CoV-2 infection has an impact on reproductive health (23). It is known that the ACE2 receptor, the key component of the renin-angiotensin system (RAS), that modulates the cleavage of angiotensin II (Ang II) and angiotensin (1–7) (Ang (1–7)) is also the functional receptor for SARS-CoV-2 virus thus allowing entrance of the virus in susceptible cells. It is also known that Ang II, ACE2 and Ang (1–7) regulate basic functions in the male and female reproductive systems. Those functions include folliculogenesis, steroidogenesis, oocyte maturation, ovulation, and endometrial regeneration in the female and in the male, testicular ACE2 may regulate testicular function and may alter sperm's contribution to embryo quality (24). There is a greater abundance of ACE2 receptors in the male reproductive system than in the female reproductive system. As a result, males are more vulnerable to the detrimental effects of SARS-CoV-2 (24). It is interesting that both parents of our patient were infected with COVID-19 at the beginning of the pregnancy, although paternal COVID-19 was assumed on clinical and epidemiological grounds only without being formally confirmed by a positive PCR test. The mother had symptoms for two weeks at the early embryogenesis stage of pregnancy with a low-grade fever lasting up to five days for which we may only assume played a pivotal pathological role in the meiotic spindle degradation (25). Some conclusions for possible harmful effects on reproductive health can be drawn from assisted reproduction studies.

In women undergoing IVF post-COVID-19 low levels of vascular endothelial growth factor (VEGF) were found, which could negatively affect the development of ovarian vasculature, reduce the supply of nutrients for the follicles, and lead to poor oocyte quality (26, 27). Moreover, a reduced level of cytokine IL-1β, which regulates folliculogenesis and atresia (28, 29), could also negatively impact oocyte quality (26). Another study compared the IVF outcomes of nine couples before and after COVID-19 infection (30). While the results of the number of oocytes retained and fertilization rates were similar, the number of top-quality embryos (TQE) was significantly lower. TQE was considered an embryo with more than seven blastomeres on day 3, ≤10% fragmentation, and blastomeres of equal size (30). Regarding fertility, the highest influence of SARS-CoV-2 infection was seen in the reduced number and quality of embryos. Chamani et al. (31) evaluated the IVF outcomes of 1881 women who underwent procedures between January and July 2020 to the control group, who underwent procedures in 2019, before the pandemic. The mean number of euploid embryos per patient was significantly lower in May and June 2020 (31, 32). This raises the possibility that, in addition to the advanced maternal age, a recognized risk factor for aneuploidies, parental COVID-19 infection may have played a role in our case of double aneuploidy.

Apart from maternal age, other genetic and environmental factors associated with non-disjunction and aneuploidy remain elusive. Further studies concerning underlying molecular mechanisms could determine causes and contributing factors of non-disjunction errors and bring broader understanding of aneuploidies. Assessing exceptional and ultra-rare cases of segregation failure like ours may be useful in improving our understanding of the general mechanisms of nondisjunction.

ETHICAL APPROVAL

Ethical approval for this case (Ethical Committee No. 2181-147/01/06/M.S.-22-02) was provided by the Ethical Committee of the University Hospital Centre Split (Chairperson Prof. M. Saraga) on May 5, 2022.

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The editors greatly appreciate the support of all reviewers whose comments and scientific evaluation of submitted manuscripts are invaluable for ensuring the scientific quality of this journal. The following distinguished clinicians and scientists acted as reviewers:

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The editors hereby express their sincere gratitude for, and their appreciation of the work done as well as the support given to this journal.