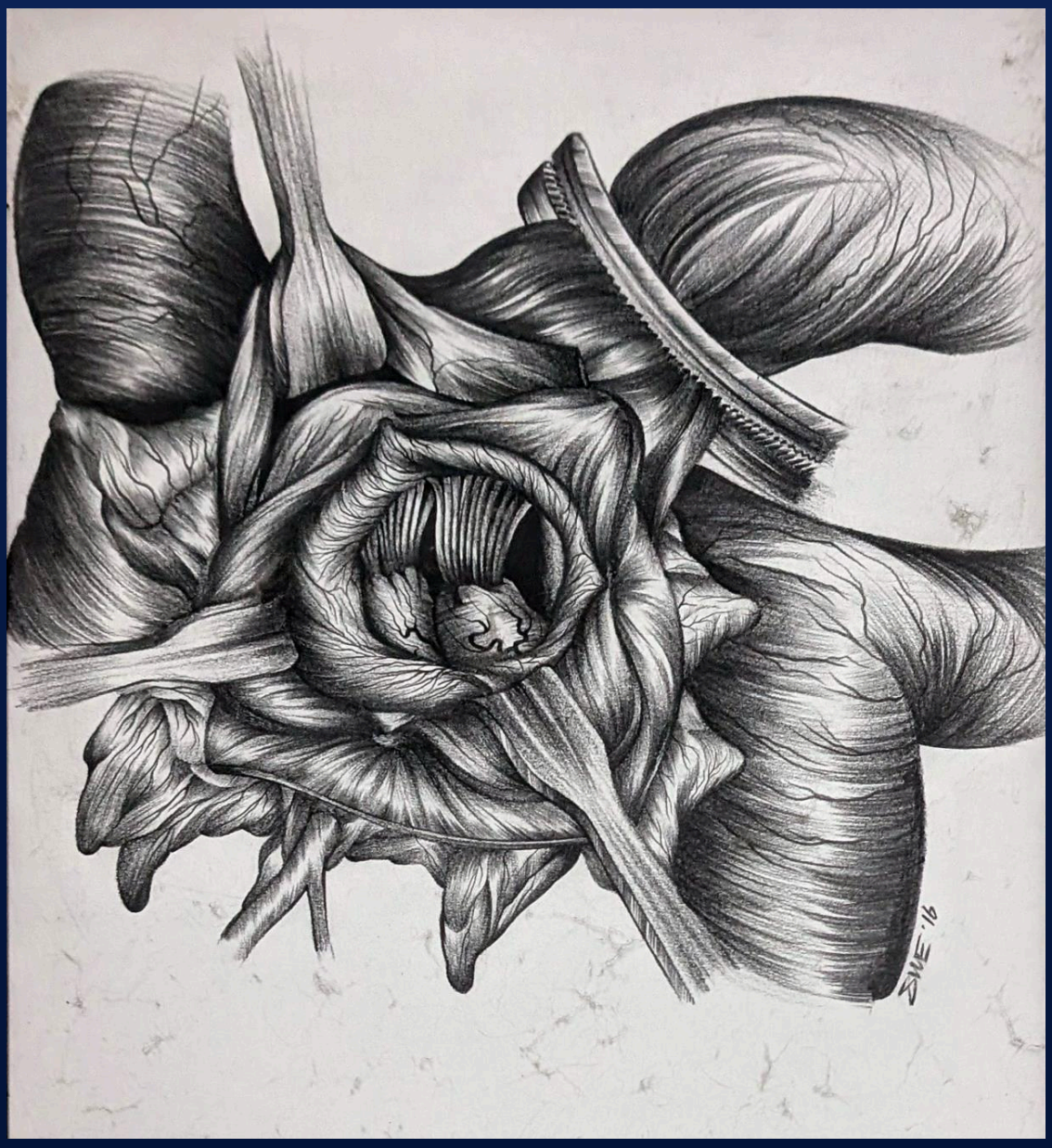
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EDITORIAL

Malta - Demographic Transition Deferred

Victor Grech

A recent paper reviewed the influence of migration on Malta's demographic transition, commenting on a debate that has moved beyond academic demography and into the public policy, economic sustainability and healthcare planning.[1] It is timely to revisit this study after international bodies have commented on the current local economic model. The paper traces fertility, population and employment trends over more than two decades, revealing a delayed of the demographic transition phenomenon at the cost of reliance on inward migration.

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COVER PICTURE

Dr Steve Micallef Eynaud is a Cardiothoracic Surgeon. He has a passion for anatomical and biological illustration, and draws on inspiration from the operating theatre as well as the natural world.

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The demographic transition model describes the shift from high birth and death rates to low birth and death rates as societies develop economically and socially. Malta, like most developed nations, has entered the later stages of this transition characterised by declining fertility, population ageing and a shrinking native workforce. What makes Malta stand out is the scale and speed at which migration has been used to compensate for this structural demographic change. Between 2000 and 2022, Malta's population increased by over 130,000 persons, the highest proportional increase in Europe, driven overwhelmingly by foreign workers and their families. At the same time Maltese fertility declined steadily, reaching the lowest total fertility rate in the European Union.

The paper's central finding shows that while total births in Malta have remained relatively stable over the study period, this apparent equilibrium conceals two divergent trends. Births to Maltese mothers have continued to decline significantly while births to non-Maltese mothers have risen sharply. Age-standardised fertility rates confirm this divergence: Maltese fertility shows a clear downward trajectory counterbalanced by non-Maltese fertility. Migration has therefore not reversed Malta's demographic transition but merely masked its most visible symptom.

From an economic perspective this strategy has been effective in the short term. Malta has enjoyed sustained growth, low unemployment and rising labour force participation including among women. Foreign workers have filled gaps across sectors ranging from construction and hospitality to healthcare, gaming and professional services. They contribute to tax revenue and social security, supporting a welfare system that would otherwise be under even more strain from population ageing. Migration has thus acted as a demographic shock absorber, delaying the workforce contraction seen in other European states.

However this solution is inherently fragile. Malta's labour market has become structurally dependent on a transient foreign workforce, with high turnover and limited long-term assimilation. A substantial proportion of foreign workers leave Malta within one to two years. This turnover places pressure on employers, inflates recruitment costs and weakens institutional memory within organisations. More importantly it exposes the economy to sudden external shocks. Any event that triggers a rapid outflow of foreign workers - whether economic downturn, regulatory change, or geopolitical instability - could precipitate a sharp contraction in output and services.

For the medical and public health community the implications are profound. Demographic transition remodels patterns of disease, service demand and healthcare delivery. Malta's ageing native population is associated with a rising burden of chronic non-communicable diseases, increased demand for long-term care and escalating healthcare expenditure. At the same time a rapidly expanding and culturally diverse migrant population brings new health needs, different disease profiles and challenges related to access, communication, and continuity of care. Healthcare systems designed around a relatively homogeneous population must adapt quickly when faced with this situation without the benefit of long-term planning.

Population growth of this magnitude also strains housing, transport, sanitation and healthcare facilities. While economic indicators may remain favourable, lived experience increasingly reflects congestion, overcrowding and service bottlenecks. In healthcare this translates into longer waiting times, increased pressure on emergency services and workforce burnout. Malta already faces shortages in key healthcare professions, a situation also partially alleviated by recruiting foreign health workers, who themselves are subject to the same turnover dynamics affecting other sectors.

Migration is not a permanent solution to demographic decline. International evidence suggests that migration can delay but not prevent the later stages of demographic transition. This is because migrant fertility tends to converge towards host-country norms over time, particularly in high-income societies. As non-Maltese residents settle, their fertility rates too are likely to fall, reproducing the same demographic dynamics currently observed among Maltese nationals. At that point Malta may find itself with both an ageing population and a diminished capacity to attract or retain foreign labour at the scale currently required.

Pronatalist policies have largely failed across developed economies with very very few exceptions. Financial incentives, tax benefits and restrictions on reproductive autonomy have produced, at best, modest and temporary effects. The decline in fertility appears to be driven less by policy levers than by deep-seated social changes including delayed marriage/commitment, extended education, women's labour market participation, housing costs and changing life aspirations. Malta is not immune to these forces and any attempt to reverse fertility decline must contend with them as well.

Malta has so far navigated demographic transition through growth and migration but this path is narrowing. Long-term sustainability will require a recalibration of the economic model with greater emphasis on productivity, skills and value creation rather than sheer population expansion. For healthcare this means investing in workforce retention, training, digital health solutions and preventive care, while ensuring that services remain accessible and equitable for a diverse population. From a medical journal perspective this paper underscores the importance of integrating demographic analysis into health planning and policy discourse.

Economists have also noted that the International Monetary Fund (IMF) data places Malta among the most densely populated territories in the world, at close to 1,800 persons per square kilometre in 2024, far exceeding the density of countries such as the Netherlands and well in the realm of city-scale systems rather than conventional nation-states. Economists have also noted that Malta's recent growth model driven by services exports and sustained by large inflows of foreign workers has effectively produced a city economy characterised by congestion pressures, infrastructure strain, housing constraints and skills mismatches. The challenges noted by the IMF are those typically associated with dense urban environments where productivity depends less on expanding headcount and more on how efficiently proximity is organised.[2]

Urban economic theory reinforces this interpretation as density becomes an asset only when supported by efficient planning and transport, functional housing markets and strong human capital formation. In their absence, density acts as a drag on wellbeing and productivity. Malta's car dependence, rising housing costs and reliance on labour-driven growth reveal a system that has arguably reached the limits of expansion. Economists thus argue that the question is not whether Malta should continue to grow but whether it can transition from labour accumulation to productivity intensification, such that infrastructure, planning, education and economic policy lead to function as a high-performing city rather than an overstretched small state.[2]

Population structure is not an abstract statistic and is a determinant of disease patterns, healthcare utilisation and system adaptability. As Malta continues to evolve demographically, clinicians, researcher, and policymakers must engage with these trends proactively rather than reactively.

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ORIGINAL ARTICLE

Potentially inappropriate prescription of hypnotic drugs in the elderly: a quality improvement project

Clarissa-Marie Zehlicke, Lisa Massa, Francesco Laybats, Saud Aldhuhli, Gareth Grech, John Cordina

Introduction and Background: Both the American Beers Criteria and the STOPP/START criteria for potentially inappropriate prescribing in older people, have recommended that benzodiazepines and Z-drugs should be avoided in older adults with a history of falls or fractures. Unfortunately, their misuse and overuse remains a worldwide epidemic. An initial audit at our rehabilitation hospital showed a significant increase in the number of patients discharged on hypnotics when compared to admission. To improve our practices we carried out a quality improvement project and a re-audit.

Methods: Multiple educational interventions were carried out. In a 2nd cycle audit we retrospectively analysed the proportion of geriatric patients admitted versus discharged on hypnotics from our rehabilitation hospital throughout 2022. We assessed for a concurrent history of falls or fractures. Results were compared to the 1st cycle.

Results: 1033 patient records were initially examined. Of those included, 132 (17.6%) were discharged on a hypnotic in the 2nd cycle compared to 183 (19.3%) in the 1st cycle. This change did not reach statistical significance. 55% had adequate documentation of prescription rationale in the 2nd cycle compared to 43% in the 1st cycle ($p = 0.046$). The majority of patients discharged on treatment had a history of fractures or falls in both cycles.

Conclusion: Prescribing practices did not improve significantly following our educational interventions. Whilst educational interventions may help with raising awareness, individually they are often not enough to instil change in practices. Multi-faceted and targeted interventions should be the focus of future attempts at improving outcomes.

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Falls and fractures remain major health concerns for the elderly, significantly impacting their morbidity and mortality. The role of clinicians in mitigating these risks through judicious prescription practices is vital. Benzodiazepines and Z-drugs, often prescribed for their sedative and hypnotic effects, have been identified as contributing factors to an increased risk of falls and fractures in this patient demographic.[1,2]

Benzodiazepines and Z-drugs act by activating the gamma-aminobutyric acid (GABA) receptors in the central nervous system. While effective for their intended uses of calming patients and sedative effects to manage insomnia, their side effects, particularly in the elderly, necessitate careful consideration and monitoring.

Both the American Beers Criteria and the STOPP/START criteria for potentially inappropriate prescribing in older people, have strongly recommended that benzodiazepines and Z-drugs should be avoided in older adults with a history of falls or fractures.[3,4] Unfortunately, their misuse and overuse remains a worldwide epidemic.[5,6]

The primary focus of our initial audit carried out at a local rehabilitation hospital, was to review the prescription patterns of benzodiazepines and Z-drugs in elderly patients admitted for rehabilitation throughout 2019. Findings showed a significant increase in the number of patients discharged on benzodiazepines and/or Z-drugs when compared to admission. Of the patients discharged on these drugs, the majority of patients had a documented history of falls and/or fractures. This highlighted the importance of better regulation of the use of hypnotics in the elderly and also highlighted the need for improved documentation of diagnosis and rationale for prescription of such drugs.[7]

The ongoing monitoring and re-evaluation of prescription practices are crucial in ensuring patient safety and improving outcomes. In order to improve our practices we implemented a quality improvement project and carried out a re-audit, not only to serve as a tool for assessment, but also as an educational and awareness-raising platform for healthcare providers.

MATERIALS AND METHODS

In order to improve patient quality of care, a number of education interventions were carried out following the initial audit results and feedback. This included 1) a verbal intervention comprising of an interactive educational lecture directed at physicians of all grades working within the department of geriatrics, 2) dissemination of an electronic educational bulletin to all physicians and allied health professionals within the department, highlighting key outcomes and recommendations 3) an oral presentation at a national conference directed specifically at local junior doctors, with distribution of results and recommendations. Throughout all 3 interventions, emphasis was placed on the need for improved documentation of prescribing rationale, the importance of initiating deprescribing of hypnotics and the significance of alternative treatment and management options.

A second cycle audit was conducted to assess for changes in prescribing and documentation practices following the aforementioned interventions. Approval was obtained from the hospital's Research Committee, Data Protection Officer and Executive Director prior to commencement.

We retrospectively analysed the proportion of geriatric patients admitted versus discharged on benzodiazepines and/or Z-drugs from the local rehabilitation hospital throughout 2022. We also assessed the percentage of patients discharged on benzodiazepines and/or Z-drugs with a concurrent history of falls or fractures. Note was made of significant comorbidities and diagnoses. Results were compared to those seen in the first cycle.

Patient Selection

The whole population of patients discharged from the local rehabilitation hospital between 1st of January 2022 and the 31stof December 2022 were included in the study. The follow patients were excluded: age less than 65 years, those discharged to the acute hospital or those who were deceased prior to discharge.

Information Resources

Two primary information sources were utilized: Electronic Case Summary (ECS) system was used in assessing medication history on admission and discharge from hospital, any documented psychiatric diagnoses, and history of falls/fractures. iSoft Clinical Manager (iCM) was used for

relevant imaging reports identifying site and year of previous fractures, and imaging requests for falls requiring assessment.

AUDIT DESIGN AND STATISTICAL ANALYSIS

Microsoft Excel Spreadsheet was used for data input and tabulation. The processed data for each patient included drugs on admission and discharge (specifically benzodiazepines and Z-drugs, including name, dose). Any change in dose between admission and discharge was noted. For those patients discharged on benzodiazepines and/or Z-drugs, additional data collected encompassed documented psychiatric history, history of falls, history of fractures, site and year of fracture/s.

Following tabulation, data analysis was carried out. Where statistical significance was sought, analysis was carried out by an independent statistician, using the chi squared test of association.

RESULTS

A total of 1033 records of patients discharged between the 1st of January and the 31st of December were initially examined (see Figure 1). The following patients were excluded: 43 were deceased prior to discharge, 81 were aged <65 years and 159 were transferred to the acute hospital; 1 patient was subsequently excluded due to an incomplete admission document. Data collection and analysis commenced on a total of 749 patients. A total of 948 patients had met inclusion criteria for the 1st cycle.

Of those included, 183 (19.3%) were discharged on either a benzodiazepine or a Z-drug in 2019 whilst 132 (17.6%) were discharged on treatment in 2022. This showed a decrease (-1.7%) in patients being discharged on treatment following the quality improvement initiatives. However, this positive change did not reach statistical significance (p = 0.377).

Whilst during 2019 there was a statistically significant increase in the prescription of benzodiazepines or z-drug from admission to discharge (+3.7%; p = 0.034), this did not recur during the second cycle, albeit there being a 3% increase of prescriptions between admission and discharge in 2022 (see Table 1).

Table 1 *Patients on treatment on admission versus discharge*

On treatment [a]	On admission	On discharge	% change on discharge
1st cycle	n= 148 (15.6%)	n= 183 (19.3%)	+3.7% (p=0.034)
2nd cycle	n= 109 (14.6%)	n= 132 (17.6%)	+3% (p=0.106)

[a]on benzodiazepine and/or Z-drug; n= total number of patients

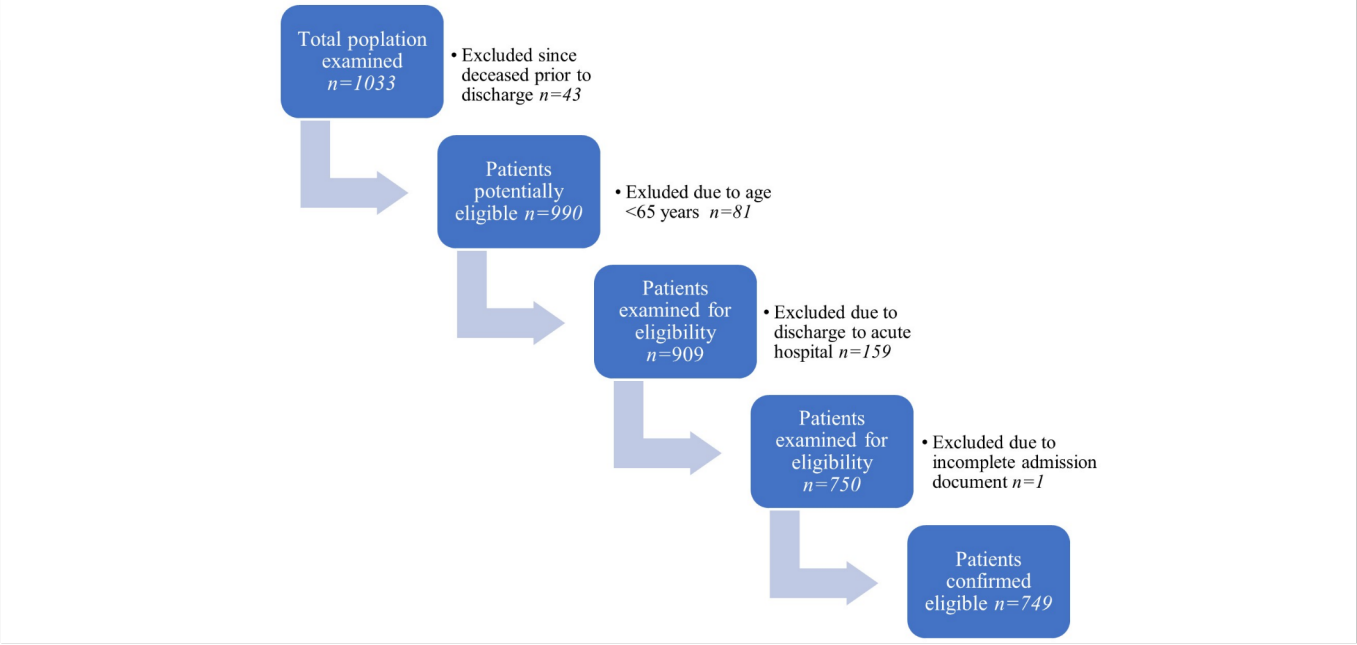


Figure 1 *Patient selection process; n= number of patients*

In 2022, from those admitted on treatment (109 patients), 39 patients (35.8%) had their dosage decreased or stopped completely; 7 (6.4%) had their dose increased; 63 (57.8%) underwent no treatment adjustment. From those not admitted on treatment (640 patients), 36 had treatment started from nil (5.6%) (see Table 2).

Of those discharged on either a benzodiazepine or a Z-drug, documentation of diagnosis improved significantly in 2022, with 55% having an adequate discharge diagnosis documented compared to 43% in 2019 (p = 0.046) (see Figure 2). Out of the 60 patients in 2022 who did not have a diagnosis documented, 43 (71.6%) were prescribed hypnotics on a purely nocturnal basis.

Anxiety (22%), depression (18%), dementia (5%), alcohol misuse (5%) and insomnia (4%) were the most commonly reported in the re-audit. Throughout both cycles, lorazepam was the hypnotic most commonly prescribed, followed by bromazepam and diazepam. Other benzodiazepines or Z-drugs prescribed were: alprazolam, clonazepam, lormetazepam, mexazolam, nitrazepam or zolpidem. The vast majority of patients discharged on treatment had a well-documented history of fractures and/or falls in both audit cycles (see Table 3).

Table 2 *Treatment (benzodiazepines and/or Z-drugs) changes from admission to discharge*

	1st cycle	2nd cycle
Treatment load decreased [a]	n=60 (40.5%)	n=39 (35.8%)
Treatment dose increased	n=11 (7.4%)	n=7 (6.4%)
Treatment started from nil	n=62 (7.8%)	n=36 (5.6%)
Treatment not adjusted	n=77 (52%)	n=63 (57.8%)

[a]*either treatment dose was decreased or treatment was stopped completely prior to discharge; n= total number of patients*

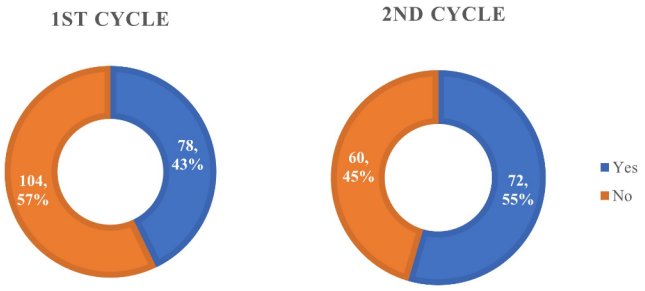


Figure 2 *Comparison between 1st and 2nd cycle audit of number and percentage of patients discharged on benzodiazepines and/or Z-drugs with a documented prescription rationale*

Table 3 *History of falls/fractures in those discharged on treatment*

	1st cycle	2nd cycle
History of fractures within last 2 years	n= 81 (44.3%)	n= 52 (39.4%)
History of fractures within last 10 years	n= 101 (55.2%)	n= 70 (53%)
History of falls	n= 121 (66.1%)	n= 101 (76.5%)

n=number of patients

DISCUSSION

Prescribing practices did not improve significantly following our educational interventions. However, a few markers from our results might indicate a positive trend in deprescribing benzodiazepines and or Z-drugs. Overall, there was a 1.7% decrease in the proportion of patients discharged on either benzodiazepines and or Z-drugs when compared to the initial audit. Moreover, whilst unfortunately there was still an increased proportion of patients discharged versus admitted on treatment, this no longer reached a statistical significant increase in the 2nd cycle (see Table 1). This might indicate an improvement, albeit not a statistically significant one. In order to reach the final desired outcome of having less patients discharged on treatment when compared to admission, we evidently still require further intervention and quality improvement.

A scoping review by Neville et al which identified 43 relevant studies, showcased a broad variety of interventions used to reduce the prescription of sedatives in hospitalized patients. On comparing studies, it was found that there was little homogeneity in the kind of interventions used. Educational interventions were the most frequently employed, either alone; for example in the form of written educational bulletins or verbal lectures, or together with other multifaceted approaches. As a singular intervention, education was not deemed as successful as when combined with other

interventions. Overall, sleep protocols and multifaceted interventions had the best outcomes.[8,9]

The one area in which we have seen a statistically significant improvement is documentation of prescribing rationale. In 2022, 55% had a diagnosis stated. This is 12% more than when compared to 2019 ($p = 0.046$). From those who did not have a diagnosis documented, the vast majority (71.6%) had their hypnotic prescribed solely on a nocturnal basis. This likely indicates that they were primarily prescribed for their sedative effects. It is a well-known fact that hospitalised patients suffer from insomnia and there are reports that up to 50% of inpatients have reported such an ailment.[10] Having better documentation of insomnia as a prescribing rationale will help policy makers understand the importance of introducing interventions such as sleep protocols and other non-pharmacological measures.

Limitations

No weight was given to failed trials of deprescribing or failed trials of alternative pharmacological or non-pharmacological treatment. We also did not evaluate for the presence of any other psychiatric treatment apart from benzodiazepines and Z-drugs. Whilst all efforts should be undertaken to avoid prescribing and to encourage deprescribing hypnotic sedatives, they remain a viable and important treatment option in some scenarios. In these cases, the potential risks have to be communicated to the patient and prescribing rationale has to be clearly documented. Long-term use should be highly discouraged.

CONCLUSION

Hypnotic sedative misuse and overuse remains a worldwide phenomenon. Whilst educational interventions may help with raising awareness, they are often not enough to instil change in prescribing and deprescribing practices. Multi-faceted and targeted interventions should be the focus of any future attempts of improving outcomes.

SUMMARY BOX

What is already known:

- It is strongly recommended that benzodiazepines and Z-drugs are avoided in older adults with a history of falls or fractures.
- The misuse and overuse of benzodiazepines and Z-drugs is a worldwide epidemic.
- The ongoing monitoring and evaluation of prescription practices are crucial in ensuring patient safety.

What are the new findings:

- A significant proportion of our patients are being discharged on benzodiazepines and/or Z-drugs.
- Educational interventions in this quality improvement project did not result in a significant improvement in prescribing practices.
- There has been a significant improvement in documentation of prescription rationale.
- Multi-faceted and targeted interventions should be the focus of any future attempts of improving outcomes.

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ORIGINAL ARTICLE

A study about the immune-related side effects at SAMOC: incidence, grades of toxicity & relation to past treatment and radiotherapy

Joanna Grech, Estelle Abela, Kelly Mifsud Taliana, Liberato Camilleri

Background: Immunotherapy targeting cytotoxic T-lymphocyte associated antigen 4 (CTLA4) and the programmed death receptor (PD-1) and its ligand (PDL1) is becoming the standard of care for many malignancies. Hence, the number of patients on immunotherapy is increasing as is the probability of developing toxicities secondary to these treatments. This project mainly aims to look at the frequency of ir-AEs at Sir Anthony Mamo Oncology Centre Malta between June 2016 and June 2020.

Method: A cohort of 177 patients were treated with immunotherapy between June 2016 and June 2020 at Sir Anthony Mamo Oncology Centre. These were assessed for immunotherapy related toxicities. These individuals were assessed for the following: age, gender, malignancy diagnosed, immunotherapeutic agents (single or combination therapy) used, systemic anticancer therapy exposure prior to ICI therapy (namely chemotherapy and TKIs), concomitant/ concurrent radiotherapy (radical and palliative RT), line of treatment at which ICI therapy was administered, the CTCAE grade and frequency of ir-AEs studied.

Results: Adverse events occurred in 43.6% of the cohort and none were recorded for 56.4%. The most common ir-AEs in our cohort were dermatological AEs (8%), thyroid-related AEs (19.4%), hepatic AEs (8%) and GI AEs (9.2%). Other data was collected and discussed further below.

Conclusion: The increasing use of immunotherapy for various cancer sites with immune checkpoint inhibitors has resulted in positive primary treatment outcomes. Secondarily, immune-related adverse events have been widely observed, as seen in the cohort assessed for all types of ir-AEs in this study.

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Immunotherapy targeting cytokine T-lymphocyte-associated antigen 4 (CTLA-4) and the programmed death receptor (PD-1) and its ligand (PDL-1) is now becoming an integral part of treatment of many malignancies. However, as the use of these immune-checkpoint inhibitors (ICI) is increasing, so is the probability that patients will develop adverse events related to these treatments. Such adverse events are often immune-related (ir-AEs) and hence are very different to those related to the more commonly used chemotherapy agents. Immune-related adverse events are new and occasionally difficult to diagnose. The incidence of ir-AEs depends on the type and regimen of immunotherapy used. The most common ir-AEs are gastrointestinal, dermatological, hepatic and constitutional symptoms[1-4]. Immune-related adverse events related to other organ systems are not as common but might be more difficult to diagnose and hence may be more severe[5].

Pooled studies have shown that combination immunotherapy (such as ipilimumab/nivolumab) in the context of advanced melanoma, 94.9% will experience at least one irAE1,[4,5]. Additionally, around 55.4% of patients will experience a grade 3/4 toxicity[5]. With single agent anti-CTLA-4 therapy incidence rate of ir-AEs was observed to be around 72% for all grades toxicities and 24% for grade 3/4 toxicities[2]. Moreover, further pooled analysis have shown that incidence of ir-AEs for anti-PD1 agents are just slightly less common when compared to anti CTLA-4; at 50-60% incidence rate for all toxicity grades and <10% for grade 3/4 toxicities[3].

This project mainly aims to look at the frequency of ir-AEs at Sir Anthony Mamo Oncology Centre Malta between June 2016 and June 2020. This study will also assess demographics, patterns of immunotherapy use and emergence of AEs, grades of reported ir-AEs as well as relationship between such adverse events and prior chemotherapy and co-current palliative radiotherapy.

METHOD

A cohort of 177 patients treated with immunotherapy between June 2016 and June 2020 treated at Sir Anthony Mamo Oncology Centre were assessed for immunotherapy related toxicities. These individuals were assessed for the following: age, gender, malignancy diagnosed, immunotherapeutic agents (single or combination therapy) used, systemic anticancer therapy exposure prior to ICI therapy (namely chemotherapy and TKIs), concomitant/ concurrent radiotherapy (radical and palliative RT), line of treatment at which ICI therapy was administered, the CTCAE grade and frequency of ir-AEs studied.

Statistical analysis using the Chi squared test was used to investigate the association between two categorical variables (i.e. previous chemotherapy use/ radiotherapy exposure and ir-AEs observed). The null hypothesis specified that there is no association between two categorical variables and is accepted if the p-value exceeds the 0.05 level of significance. The alternative hypothesis specifies that there is a significant association between the two categorical variables and is accepted if the p-value is less than the 0.05 criterion.

RESULTS

A cohort of 177 patients was reviewed. 72.3% of the group were male and 27.7% were female. The median age was 63 years (range 21-82 years). The majority of the patients were in the 61-70 age group (40.6%) followed by the 51-60 age group (25.4%) and more than 70 age group (21.5%). Demographics can be seen in Table 1.

Immunotherapy was most commonly used in lung tumours, renal tumours, cutaneous melanoma and head and neck malignancies. Immunotherapy was

Table 1 *Demographics*

	n	%
Male	128	72.3%
Female	49	27.7%
20-30 years	4	2.3%
31-40 years	4	2.3%
41-50 years	14	7.9%
51-60 years	45	25.4%
61-70 years	72	40.6%
More than 70 years	38	21.5%

Mean = 62.16 yrs, Median = 63 yrs, Range = 21-82 yrs

used to a lesser extent in other malignancies as shown in Table 2. The majority of patients receiving immunotherapy at SAMOC were given this in the context of metastatic disease (87.6% in the metastatic setting versus 12.4% in the adjuvant/ locally advanced setting).

Table 2 *% Malignancies in which ICI used*

	n	%
Colon	2	1.1%
Head and Neck	19	10.7%
Renal	31	17.5%
Bladder	12	6.8%
Lung	68	38.4%
Uveal melanoma	4	2.3%
Cutaneous Melanoma	20	11.3%
Choroidal melanoma	4	2.3%
Mucosal melanoma	1	0.6%
HCC	4	2.3%
Cholangiocarcinoma	1	0.6%
Testes	1	0.6%
Penile	1	0.6%
Astrocytoma	1	0.6%
Breast	7	4.0%
Mesothelioma	1	0.6%
Oesophagus	1	0.6%
SCC unknown primary	1	0.6%

Table 3 *Type of immunotherapy*

n	%	
Pembrolizumab	81	45.8%
Nivolumab	78	44.1%
Ipilimumab & Nivolumab	16	9.0%
Ipilimumab	2	1.1%

Table 4 *Number of cycles*

	n	%
1-5	68	38.7%
6-10	50	28.4%
11-15	25	14.2%
>16	33	18.7%

With regards to patterns of immunotherapy administration and emergence of ir-AEs, only 23.7% of patients received immunotherapy as first line treatment while in 76.3% immunotherapy was used as second line treatment. Single agent pembrolizumab (45.8%) and nivolumab (44.1%) were the most commonly used ICIs and combination ipilimumab/nivolumab was used in 9% of cases. Table 3 shows a summary of this.

The majority of patients in our cohort has 15 cycles of treatment or less (1-5 cycles 38.7%, 6-10 cycles 28.4% and 11-15 cycles 14.2%). 1.7% had more than 41 cycles. The median number of cycles was 7 (range 1-54). This is summarised in Table 4. Adverse events occurred in 43.6% of the cohort and none were recorded for 56.4%. Ir-AEs in this study occurred mostly in cycles 1-5 (24.9%) followed by in cycles 6-10 (14.1%). Median number of cycles at which ir-AEs occurred was at cycle 4 (range 1-31), as shown in Table 5.

Table 6 shows the frequency of ir-AEs and related grades in our cohort over a 3-year period. The most common ir-AEs in our cohort were dermatological AEs (8%), thyroid-related AEs (19.4%), hepatic AEs (8%) and GI AEs (9.2%). Respiratory AEs, fatigue and renal AEs were slightly less common at 6.9%, 3.4% and 4% respectively. Pituitary, neurological, rheumatological, ocular and haematological AEs as well as diabetes were very uncommon. Grade 1 AEs were the most common followed closely by Grade 2 events. Grade 3/4 events were uncommon.

Additionally, we also looked at the relationship between prior chemotherapy/Tyrosine-kinase inhibitors (TKIs) and an increased number of adverse

Table 5 *Number of cycles prior to event*

	n	%
1-5	44	24.9%
6-10	25	14.1%
11-15	2	1.1%
16-20	3	1.7%
21-25	1	0.6%
26-30	1	0.6%
31-35	1	0.6%
No adverse events	100	56.4%

Table 6 *Frequency of ir-SEs and related grades*

Organ	IR-AEs	n	%	G1	G2	G3	G4
Constitutional	Fatigue	6	3.4%	1.7%	1.1%	0	0.6%
Skin	All	15	8.6%				
	Rash	12	6.8%	2.8%	4.0%	0	0
	Pruritus	1	0.6%	0.6%	0	0	0
	Other	2	1.2%	0.6%	0.6%	0	0
Thyroid	All	32	19.4%				
	Hypothyroid	26	14.8%	11.4%	3.4%	0	0
	Hyperthyroid	6	4.6%	4%	0.6%	0	0
Pituitary	All	4	2.4%	0.6%	1.2%	0	0.6
Pancreas	All (DMT1)	1	0.6%	N/A	N/A	N/A	N/A
Liver	All (hepatitis)	14	8%	4%	2.3%	1.1%	0.6%
GI	All	16	9.2%				
	Diarrhoea	9	5.1%	2.8%	0.6%	1.7%	0
	Blood PR	1	0.6%	0.6%	0	0	0
	Abdominal Pain	4	2.3%	0.6%	1.1%	0.6%	0
	Vomiting	1	0.6%	0	0	0.6%	0
	Other	1	0.6%	0	0.6%	0	0
Lung	All	12	6.9%				
	SOB	3	1.7%	0	1.1%	0.6%	0
	Cough	5	2.9%	0	2.3%	0.6%	0
	Radiological Changes	3	1.7%	0	1.1%	0.6%	0
	Other	1	0.6%	0	0	0.6%	0
Neurological	All	2	1.2%	0	0.6%	0.6%	0
Cardiac	All	0	0	0	0	0	0
Rheumatology	All	4	2.3%	0	1.7%	0.6%	0
Kidney	All	7	4%	1.1%	2.3%	0.6%	0
Eyes	All	3	1.7%	0.6%	1.1%	0	0
Haematology	All	2	1.2%	0	0.6%	0	0.6%

events. There seemed to be no increased number of AEs in the group that had prior treatment. Conversely, there was an association between the patients who were on co-current palliative radiotherapy and an increased number of AEs. Pt who were on co-current radiotherapy seemed to have an increased number of AEs (p=0.028).

Finally, with regards to effect of AEs on continuation of treatment, in 85.9% treatment was stopped only in view of disease progression or death unrelated to immunotherapy. Treatment was stopped due to ir-AEs in 9% of patients in our cohort and in 5% treatment was stopped until AE was treated and then restarted.

DISCUSSION

Immune check point inhibitors (ICIs) have revolutionised cancer therapy. In comparison to conventional chemotherapy or even tyrosine kinase inhibitors, ICI's have a favourable side effect profile. However, their mode of action may lead to immune-related adverse effects. Our study delves into the prevalence of these effects, and any correlation between their occurrence on a background of radiation exposure or even use of prior SACT (systemic anti-cancer therapy).

Mode of Action of Immunotherapy

CTLA-4 and PD-1 receptors are centrefold in the ligand-receptor interactions between the antigen presenting cells (APCs) and T-cells modulating the T-cell response to the antigen. CTLA-4 will control the state of activation of the effector T-cells and inhibits cytokine production therefore initiating tolerance. CTLA-4 also negatively modulates helper T-cell activity thus suppressing T regulatory cells activity. PD-1 induced on dendritic cells, macrophages, activated and T-regulatory cells, extensively modulates effector T-cell function within normal tissue and tumours.[6] PD-L1 is also expressed on the aforementioned cells including tumour cells to escape anti-tumour responses. It acts as a pro-tumorigenic factor by activating survival and proliferative signalling pathways when binding to its receptors.[7]

Monoclonal antibodies (mABs) can block the ligand receptor interactions of immune check points. CTLA-4 mAB Ipilimumab acts by blocking the binding of CTLA-4 on T-cells to ligands CD80/86 on tumour cells. Pembrolizumab and Nivolumab bind to the inhibitory receptor PD-1 consequently inhibiting PDL-1/ PDL-2 interactions and hindering T-cells cytotoxic mechanisms.[6] Atezolizumab, Avelumab and Durvalumab are PD-L1 inhibitors bind to PD-L1 disrupting the PD-1 axis reversing T-cell suppression enabling endogenous anti-tumour activity with long-term responses in a spectrum of cancers.[8]

Ir-AEs incidences and Our Cohort

ICI adverse events (AEs) are known as immune-related (IR) AEs which are graded in line with the 5th version of the Common Terminology Criteria for Adverse Events (CTCAE).[9] This study assesses patient demographics, therapeutic indications of immunotherapy, grade and emergence of ir-AEs in addition to the relationship between these and concurrent radiotherapy. Ir-AEs incidences were compared to those listed in the Management of toxicities from immunotherapy: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up (ESMO CPG 2022).

Immunotherapy was mostly used in the age groups whereby cancer areas were treated as per guidance which was prevalent in the male subgroup. This was also more prevalent in the metastatic setting.

A higher incidence rate of endocrinopathy namely thyroid dysfunction was seen, with hypothyroidism occurring in 14.8% (versus 9%, ESMO CPG 2022)and hyperthyroidism in 4% (versus 1-2%, ESMO CPG 2022) of patients in comparison to diabetes mellitus which occurred in <1% (versus 1-2%, ESMO CPG 2022) across ICI regimens, with similar rates of hypopituitarism of 1.8% (~1% with antiPD-1, 2-6% with anti-CTLA-4 and 9-10% incidence with combined therapy, ESMO CPG 2022) . In addition, ocular events such as anterior uveitis were more frequent in our cohort at 1.2% (versus <1%, ESMO CPG 2022). On the contrary, less commonly reported events were: skin toxicity, with 8% of individuals affected (versus >50% for all grades, ESMO CPG 2022), gastrointestinal mainly diarrhoea in 5.1% (versus ~35% with anti-CLTA-4 mAB, ~10% with antiPD-1 and ~32% with combination immunotherapy ipilimumab and nivolumab, ESMO CPG 2022) requiring colonoscopy in two patients with cessation of immunotherapy.[10] Similar incidences were observed for hepatitis 8% (5-10%, ESMO CPG 2022) with deranged liver function tests on a background of progression of metastatic liver disease whereby four patients were treated with steroid therapy for hepatitis. Furthermore, ir-pneumonitis with cough (2.9%) and CT related changes occurred in 1.7% of patients with CTCAE grade 2 and grade 3 toxicity were observed (in comparison to 2-4%, ESMO CPG 2022). A global lower incidence of IRAE's was observed in this cohort of patients, this could be a result of underreporting or underdiagnosing the individual organ dysfunction. However, our cohort numbers correlate with those reported by ESMO in terms of prevalence.

Ir-AEs were observed in 24.8% of individuals who had received chemotherapy prior to immunotherapy. Chemotherapy is known to potentiate the effects of immunotherapy as SACT may directly stimulate antitumour immunity.[12] As a result of this one can stipulate that this activity may also catalyse the adverse effects seen in patients treated with immunotherapeutic agents.

With respect to radiation exposure (in the form of external beam radiotherapy), a pooled analysis of trials by Ansher et al has not shown an increased risk of IRAE's in patients receiving an ICI within ninety days following radiotherapy versus those who had not received radiation.[11] An explanation for this is that adverse effects tend to occur in the organ that received radiation, rather than a systemic reaction to the combined effects of radiotherapy with concomitant ICI therapy. On the contrary, a statistically significant association between number of side effects and concurrent radiotherapy was observed in our cohort ($p = 0.028$). The number of side effects increases with concurrent radiotherapy. This could be related to the smaller number of individuals studied augmenting the correlation seen. In addition, patients receiving radiotherapy may still experience side effects from this independent of the systemic therapy received.

Limitations

Our study may be limited by the smaller number of patients observed retrospectively. In addition, the short observation period (from 2016 to 2020) may have not completely reflected the preponderance of ir-AEs in our local cohort of patients. Immunotherapy is now being used in various malignancies including the neo-adjuvant and adjuvant settings in sites such as melanoma, lung cancer and breast cancer, which could possibly result in an increased incidence in ir-AEs recorded locally to date.

CONCLUSION

The increasing use of immunotherapy for various cancer sites with immune checkpoint inhibitors has resulted in positive primary treatment outcomes. Secondarily, immune-related adverse events have been widely observed, as seen in the cohort assessed for all types of ir-AEs in this study.

The effective treatment of ir-AEs is dependent on their early recognition and prompt intervention with immune suppression and/or immunomodulatory strategies for the affected organ system and the grade of toxicity. Specialist physicians, nurses and pharmacists that manage ir-AEs should be involved early on, possibly requiring inpatient management in serious (≥ grade 4) or grade 3 ir-AEs that are refractory or unsatisfactorily responsive to outpatient therapy. Such an intervention can also hasten clinical work-up and therefore prevent complications from potentially life-threatening adverse effects. Setting up of a local immunotherapy service guided by the Clinical Oncologist and the Nurse Specialist may facilitate better management of patients on ICIs.[12]

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ORIGINAL ARTICLE

Stone Free Rates following Ureteroscopy at Mater Dei Hospital

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Introduction: Renal calculi pose a huge burden on health care systems and their rising prevalance has led to an increased reliance on surgery, particulary for upper urinary tract stones. Techincal advancements has made ureterscopy (URS) a mainstay treatment for both renal and ureteric stones. Despite its increase use, reported stone free rates following URS vary considerably owing to the different imaging modalites used and the different defintions of stone free. The aim of this audit is to assess the stone free rates of patients who underwent a URS at Mater Dei Hospital, Malta.

Methods: All patients who underwent a URS for stones between January 2019 and December 2020 were included. Data was collected retrospectively by reviewing records on two main online hospital systems: Isoft Clincal Manager (ICM) and Picture Archiving and Communication System (PACS).

Results: A total of 588 patients were selected for data analysis after applying inclusion/exclusion criteria. CT KUB was most common imaging modality for both diagnosis (83%, n=496) and follow-up (64%). At first follow-up, 418 patients (71%) were stone free when using the 5mm cut off, while 298 patients (50%) were stone free when using the 2mm criteria. At first follow-up, 209 (66%) of pre-stented patients were stone free when using the 5mm cut off, while 148 (47%) patients were stone free when using the stricter 2mm criterion. Patients with ureteric stones had better stone free rates at first follow-up at both 2mm (0.01) and 5mm cuts offs (p=0.013). Stone free rates were worse for patients who had stones higher up in the calyces of the kidney (p=0.01). The distribution of HU was the same across all categories of stone free at first follow-up when considering 2mm cut off (p=0.544). A total of 22 patients sufferred post-operative complications. 13 of these were re-admitted within 30 days of surgery and one patient died.

Conclusion: Different stone parameters as well as different imaging techniques influence stone free rates. Ureteroscopy is now the most commonly performed stone removing procedure. Efforts should be made to render patients stone free, as this will reduce the requirements for future secondary interventions.

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Renal calculi pose a huge burden on health care systems and their rising prevalance have been attributed to increased rates of diabetes, obesity and metabolic syndrome.[1] Prevalence rates are particularly high (>10%) even in countries with a high standard of living such as Sweden or the USA.[2] This increase in prevalance has led to an increased reliance on surgery for the treatment of such stones, especially those within the upper urinary tract.

With the availability of both rigid and flexible ureteroscopy (URS), all areas the of the urinary tract can be readily accessed. Technical advancements including better optical quality, endoscope miniaturisation, improved deflecton mechanism and the introduction of disposables has made URS a mainstay treatment for both renal and ureteric stones.[3] Despite its increased use, there has been alot of variations in the reported stone free rates following URS. These discrepancies are often the result of inconsitencies in the definition of 'stone free'. This is often attributed to variations in the type of imaging used in assessing for residual stones postoperatively, as well as the timing of the assessment. Other potential factors that might account for such variations of stone free rates include size and postion of stone.[4]

AIM

The aim of this audit was to assess the stone-free rates of patients with renal and ureteric stones who were treated with URS at Mater Dei Hospital. The primary outcome is the stone free rate after URS at first and last follow-up using 2 and 5mm cut-off points. Other outcomes include type of diagnostic imaging modality used, stone parameters, interval to follow-up imaging, re-admissions and post-operative complications.

MATERIALS AND METHODS

The audit was conducted between January 2019 and December 2020. A proforma with no patient identifiers was used and all data was anonymised; stored internally on a hospital p-drive folder; and deleted after audit completion. Data protection clearance and ethics approval were obtained prior to the start of data collection.

All individuals who underwent a URS for stones over this two-year period were included. Patients were excluded if they were lost to follow-up; if there was any incomplete information or missing data; if radiology images were not available on the online hospital system; and if the URS was not primarily done for stone extraction. For those who met the inclusion criteria, data was collected in a retrospective fashion by reviewing records on two main online hospital systems: Isoft Clincal Manager (ICM) and Picture Archiving and Communication System (PACS).

Stones were classified according to size, position, density and compostion. Stone size was measured in millimeters (mm) using the measurement tool and stratified into groups of up to 5mm, 6-10, 11-15, 16-20, 21-25, 26-30 and >30mm in largest diameter. Stone location was defined according to anatomical position: upper, middle or lower calyx; renal pelvis, pelvic-ureteric junction (PUJ), upper, middle or lower ureter; and vesico-ureteric junction (VUJ). Stone density was given in hounsfield units (HU) and estimated using an ellipticial annotation tool that measures density over the particular area of interest. The number of patients who were stented prior to the URS was recorded and stone free-status was determined at first imaging following the URS. Stone free status was defined as clinically insignificant stone fragments 5mm or less. A stricter definition of stone free was also defined as stone fragments 2mm or less. Stone parameters were analysed using the Chi-squared test for catergorical variables while the Mann-Whitney U test was used for continous variables. A p value of less than 0.05 was considered to be statistically significant.

When available, laboratory renal calculi analysis was used to establish stone type and composition. Length of stay was defined as the time from completion of the URS to discharge from hospital. Electronic case summaries were manually screened to check for any re-admissions and record any associated post-surgical complations. The severity of the post-operative complications was graded using the Clavien-Dindo Classification. In cases where prolonged hosptial stay was multifactorial, the predominant reason for the prolonged stay was taken as the cause.

RESULTS

Between January 2019 and December 2020 a total of 739 patients underwent a URS at MDH. After applying inclusion/exlcusion criteria 588 patients were selected for data analysis. Of the 151 exluded patients, 86 patients had URS which was not primarily done for stones, while the rest either had inadequate follow-up/incomplete information or radiology investigations were not available.

The most common diagnostic imaging modality was a non-contrast CT KUB (83%, n=496) followed by a contrast CT Abdomen/Pelvis (7%, n=43). Again, CT KUB was the preferred modality to assess stone burden after URS (64%, followed by ultrasound of the kidneys, 21%). The mean time to URS from imaging was 78.6 days while the mean time to first follow-up imaging after URS was 138.1 days.

The mean length of stay was 1 day (minimum of LOS of 0 and maximum of 31 days). At first follow-up, 418 patients (71%) were stone free when using the 5mm cut off. This number was reduced to 298 patients (50%) when using the 2mm criteria.

A total of 316 patients had a ureteric stent insertion prior to the URS. At first follow-up, 209 (66%) of pre-stented patients were stone free when using the 5mm cut off, while 148 (47%) patients were stone free when using the stricter 2mm criterion (Figure 1 and Figure 2). For those who did not have prior stent insertion, a lower percentage of 49% and 36% were stone free at first follow-up when using the 5mm and 2mm cut offs respectively. The difference between pre-stented and non-stented stone free rates was statistically signficant (p=0.003).

A total 56% (n=329) of the treated stones occurred inside the ureter, with the distal third of ureter being the most common location (n=136, 23%), followed by the proximal third (n=128, 21%). Patients with ureteric stones had better stone free rates at first follow-up at both 5mm (p=0.013) and 2mm cut offs (0.01) (Figure 3 and Figure 4). Stone free rates were worse for patients who had stones higher up in the calyces of the kidney (p=0.01).

The average stone density was 592 HU, with the majority of stones possessing a density which was less then 1000 HU ((n=242 <500 HU), (n=233, 501-1000 HU), (n= 57 >1000 HU)) (Figure 5). The distribution of

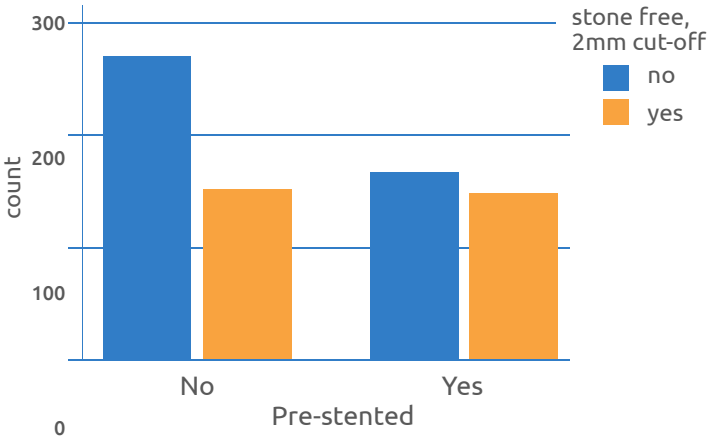


Figure 1 *Relationship between pre-stenting and stone free status using the 2mm cut off*

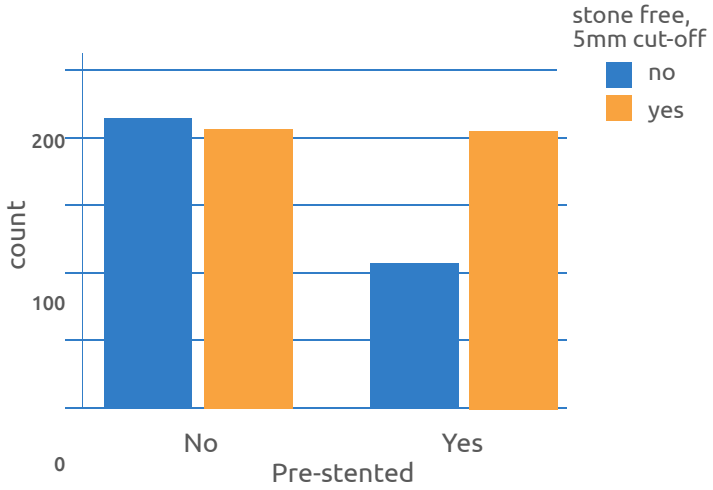


Figure 2 *Relationship between pre-stenting and stone free status using the 5mm cut off*

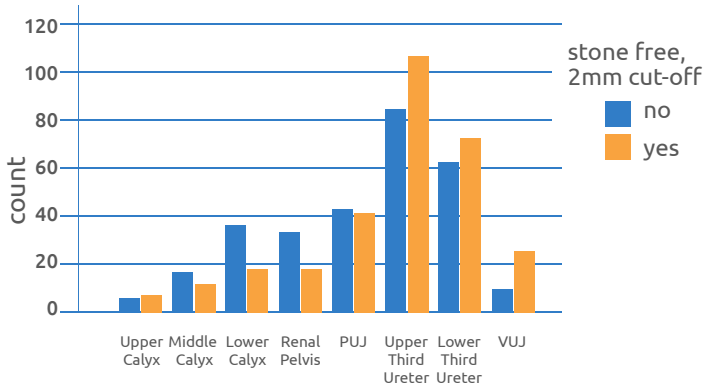


Figure 3 *Stones free rates on first follow up using the 2mm cut off*

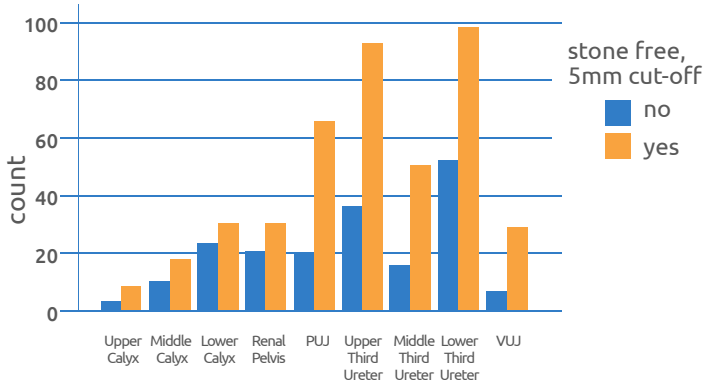


Figure 4 *Stones free rates on first follow up using the 5mm cut off*

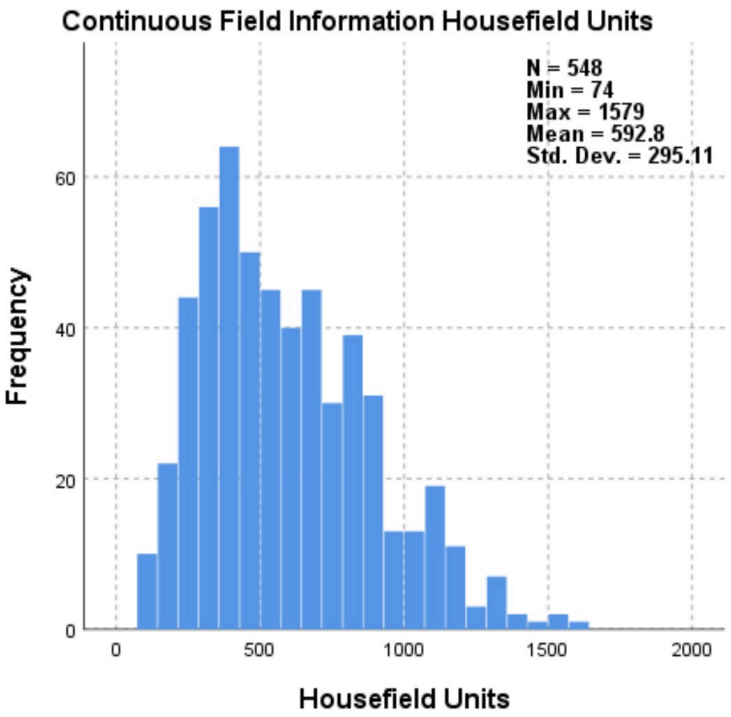


Figure 5 *Stone Densities*

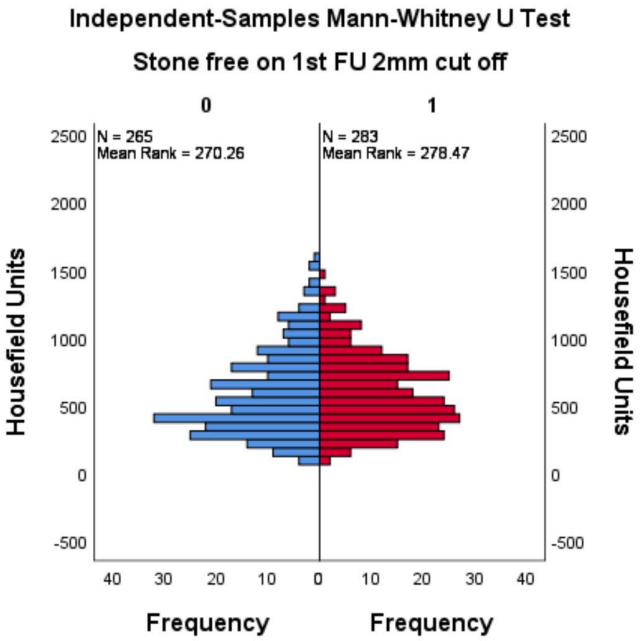


Figure 6 *Relationship between density and stone free status using 2mm cut off*

HU was the same across all categories of stone free at first follow-up when considering 2mm cut off (p=0.544). Stones with a HU of less than 1000 were associated with better stone free rates, however, this finding was not statistically significant (Figure 6). Median stone density was 534 Hounsfield units (HU) in stone-free patients and 526 HU in non–stone-free patients. The median difference in stone density was 8 HU, with a 95% bootstrap confidence interval ranging from −64 to 96 HU, indicating no clinically meaningful difference between groups. Consistent with this, the effect size was negligible (Cliff's δ = 0.03; 95% CI −0.07 to 0.13), suggesting that stone density alone had limited discriminatory value for predicting stone-free outcome at first follow-up. The most common stone size ranged between 6-10mm (n=339, 58%) followed by 11-15mm (n=114, 19%). Stone free rates increased with decreasing stone size (p=0.033).

22 patients sufferred post-operative complications (severe sepsis [n=9], septic shock [n=5], urinary tract infection [n=6], ureteric obstruction [n=2]. 13 of these were re-admitted within 30 days of surgery and one patient died. Taking into account the Clavien-Dindo classification of surgical complications there were five cases of Clavien-Dindo 4 complications and one 5 complication. This corresponds to an overall complication rate of around 4%, which is lower than rates reported in the existing literature. However, complication data were collected retrospectively from electronic case summaries, and therefore minor or self-limiting events that were not documented in formal clinical records may have been missed. As such, the true complication rate may be underestimated, particularly for low-grade complications not requiring intervention.

DISCUSSION

URS success rates quoted in literature vary considerably from 59% to 95%.[5] However, these outcomes can be misleading due to different variety of imaging techniques used and different defintions of stone free rates.

In patients with suspected acute urolithiasis, a non contrast enhanced CT has become the standard imaging and is significantly more accurate than IVU or US.[6] A non-contrast CT can determine stone diameter, density, skin to stone distance and surrounding anatomy, all of which play an important part in selection of treatment modality.[7] In pregnant women, US is the preferred method of imaging for suspected renal colic. MRI can also be used, usually a second line-option, but also to define level of obsruction in the urinary tract and to visualise stones as a filling defect.[8]

Owing to its minimally invasive nature, URS generates alot of in situ fragmentation, hence, residual fragments are not always passed. This finding contrasts traditional open surgery, where stones were removed in toto. The term clinicially insignifcant residual stone fragments started being used for asymptomatic stones which were less then 5mm in size, and this metric was adopted by many studies when reporting stone free rates after URS. This idea started being questioned when a large proportion of patients who had insignifcant residual stones developed significant symptoms or required additional interventions during their follow-up period.[9] In view of this, we used both 5mm and 2mm cut-off points, as we thought that the 2mm cut-off might be more appropriate for establishing the true clinical significance of stone-free.

Elective stenting is not necessary prior to URS, however, a recent meta-analysis [10] found that pre-stenting may improve stone free rates of URS for renal stones but not ureteric stones. This is thought to be due to a dilatation effect of a double J stent which relieves obstructed ureters and improves access to the upper tracts at the time of the URS. Although it might improve ureterscopic management of stones and placement of ureteric access sheath, pre-stenting does not significantly decrease the risk of intra-operative complications.[11] Moreover, stenting causes the patient to experience stent-related symptoms and might be associated with higher patient morbidity and costs.[12] Routine stenting after uncomplicated URS is also not recommended and stents should only be inserted in patients who are at risk of adverse events such as bleeding, perforation, UTIs, residual stones and ureteral trauma.[13]

Size is an important parameter when considering stone free rates after URS. With increasing stone sizes, obtaining complete clearance during URS is more challenging and sometimes patients might required multiple (staged) URS to achieve acceptable outcomes.[14] Also, when actively picking up residual stones using dormia basket after the primary stone has been fragmented with laser, smaller stone fragments might be easily missed or difficult to retrieve.

Stone location might also affect complete stone clearance. Treatment of lower pole calculi might be hampered by the limited flexion of the ureterscope that occurs when a laser fiber or a dormia basket is in place. In addition to possible scope damage, it might also reduce stone clearance.[15] Research has shown significance of HUs with regards to stone free rates after URS.[16] However, in our audit stone density did not really discriminate outcomes. This showed that outcomes after URS are multifactorial and influenced by other factors beyond stone density.

While in many centres worldwide URS is a day case procedure [17], locally, the majority of patients who undergo a URS for stones are kept for an ovenight stay and discharged the day after, following trial without catheter (TWOC). This is reflected in a mean length of stay of 1 day. The timing of follow-up imaging may also influence stone free rates. Small residual fragments may pass days or weeks following URS while new calculi may form weeks or months after surgery. Late follow-up imaging may increase the risk of potential contamination form new stone formation. Prolonged operation times are associated with increased risk of complications following URS, and efforts should be made to keep operation time below 90 minutes.[18]

A limitation of this audit is the small number of complications recorded. This might be related to the retrospective nature of this study which can lead to recall bias and potential missing or inaccurate data from exisitng records. Another possible cause might be linked to the Clavien-Dindo classification system which might have not captured all possbile URS complications, leading to an underreporting of minor self-limiting complications. We also focused on complications occuring during the first 30 days post-operatively. This limited follow-up might have missed the development of delayed complications, such as ureteral strictures, which can occure weeks or months after the procedure.

CONCLUSION

URS is a widely used stone removal procedure.[3] Efforts should be made to ensure that the patient is stone free, decreasing the need of future surgical interventions. Different stone parameters and different imaging techniques affect stone free rates after URS. Different defintions of stone free might also impact stone free status , hence a standard defintion of stone free should be used in future studies.[19]

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ORIGINAL ARTICLE

Knowledge of Spirometry in Foundation Doctors and Basic Specialist Trainees in Medicine

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Background: Basic interpretation of spirometry is important to any clinician, since respiratory disease is one of the most encountered in everyday clinical practice.

Methods: The aim of this audit is to assess the basic knowledge (via a questionnaire) of spirometry interpretation by foundation doctors and basic specialist trainees working in medicine, in Mater Dei Hospital, Malta. This included the interpretation of the values for Forced Expiratory Volume in one second (FEV1), Forced Vital Capacity (FVC) and their ratio.

Results: The maximum score from the questionnaire was 9. A total of 21 FYs and 30 BSTs participated in the data collection. The mean score for the FY group was 6.8, the least score being 4 (obtained by 1 participant) and the highest being 8 (obtained by 6 participants). The mean score for the BST group was 6.8 as well, the least score being 5 (obtained by 6 participants) and the highest being 9 (obtained by 2 participants).

Conclusion: Although relatively satisfactory scores were obtained from the two groups, it is still concerning that a significant amount of participants answered basic questions incorrectly; these basic principles being important in the interpretation of a spirometry result. Further education is required to standardise the knowledge on the basic interpretation of spirometry. Interpretation of lung function testing is important as this could affect management, including organising further investigations or identification of important reversible obstructive lung diseases such as Asthma.

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Spirometry, also known as 'lung function testing', is a simple non-invasive technique by which lung volumes can be measured in patients with suspicion of known respiratory disease[1]. Basic interpretation is important to any clinician, since respiratory disease is one of the most encountered in everyday clinical practice.

The aim of this audit is to assess the basic knowledge of spirometry interpretation by Foundation Year Doctors (FYs) and Basic Specialist Trainees (BSTs) working in medicine, in Mater Dei Hospital, Malta. The knowledge assessed would include interpretation of the values for Forced Expiratory Volume in one second (FEV1), Forced Vital Capacity (FVC) and their ratio; along with interpretation of reversibility testing (indication and positive/negative results). The doctors will also be assessed on the knowledge of contra-indications for spirometric testing.[2]

MATERIALS AND METHODS

A questionnaire was used to assess the knowledge of FYs, some of whom were working in the Pre-Operative Assessment clinic, and Medical BSTs. All participants were informed regarding the aim of the audit beforehand and were asked whether they accept to be assessed in the audit. All data collected is anonymised.

The questionnaire is in accordance with clinical practice guidelines on spirometry indication and interpretation[3]. One point will be scored for each correct answer in the questionnaire. The results were then assessed for any statistical significance using the independent t-test, with statistical significance being met if the p value was <0.05.

The questions that were asked during the data collection can be found in **Supplemental File 1**.

RESULTS

A total of 21 FYs and 30 BSTs participated in the data collection. During the data collection for this audit, a new update was issued by the European Respiratory Society and American Thoracic Society regarding interpretation of variability in FEV1/FVC, with the new guidance suggesting bronchodilator positivity when there is a 10% relative to the predicted FEV1/FVC, rather than a 12% change (or 200ml change) between pre- and post-bronchodilator values[5]. In view of this update, questions in our audit with regards to lung reversibility (Questions 7 and 10) were discarded, to avoid confusion with regards to accurate responses. Therefore, the maximum score one could have obtained was 9.

Table 1 depicts a summary of the results for the Foundation Doctor Group.

The mean score for the FY group was 6.8, the least score being 4 (obtained by 1 participant) and the highest being 8 (obtained by 6 participants). The least correctly answered question was Question 11, which asks regarding the recommended spirometry time for an accurate result.

FYs who have previously worked in a Respiratory Rotation (n=10) scored a mean of 6.6, whilst those who didn't (n=11) had a mean score of 7. When comparing the means of the two groups, the results were not statistically significant when using an independent two-tailed t-test (p= 0.43).

Table 2 depicts a summary of the Medical BSTs results.

The mean score for the BST group was 6.8 as well, the least score being 5 (obtained by 6 participants) and the highest being 9 (obtained by 2 participants). The least correctly answered question was also Question 11, with 22 participants answering incorrectly.

Although the mean scores for both groups were 6.8, there was no statistical significance when being assessed with an independent two-tailed t-test (p=0.90).

DISCUSSION

The 2005 Technical Standards for Spirometry were updated in 2019 by a joint task force between the European Respiratory Society (ERS) and the American Thoracic Society (ARS)[4]. The changes reflect the improvements in instrumentation and research to ensure international standards are met.

Spirometry measures the maximal volume or flow of air that can be inspired and expired. It helps diagnose respiratory conditions and assess general respiratory health, effect of a disease and therapeutic interventions on lung function, airway responsiveness, pre-operative risk and prognosis. The

Table 1 *Results of the Foundation Doctor Group. Questions 7 and 10 being discarded.*

Participant Number	At POAC or Ward?	Surgical or Medical Rotation	Ever worked in a Respiratory Rotation?	Q1	Q2	Q3	Q4	Q5	Q6	Q8	Q9	Q11	Result
1	Ward	Surgery	No	✔	X	✔	✔	✔	X	✔	X	✔	6
2	Ward	Medicine	Yes	✔	✔	✔	✔	✔	✔	X	X	✔	7
3	Ward	Medicine	No	✔	✔	✔	✔	✔	✔	X	✔	X	7
4	POAC	Surgery	No	X	✔	X	✔	✔	✔	X	✔	X	5
5	Ward	Medicine	Yes	✔	✔	✔	✔	✔	✔	X	✔	X	7
6	POAC	Surgery	No	✔	✔	✔	✔	✔	✔	✔	✔	X	8
7	Ward	Medicine	Yes	✔	X	✔	✔	✔	✔	✔	✔	✔	8
8	POAC	Surgery	No	✔	X	✔	✔	✔	✔	✔	✔	✔	8
9	Ward	Medicine	Yes	X	X	✔	✔	X	✔	✔	X	X	4
10	Ward	Medicine	No	✔	✔	✔	✔	✔	X	✔	X	X	6
11	Ward	Medicine	Yes	✔	✔	✔	✔	✔	X	X	✔	✔	7
12	Ward	Surgery	No	✔	✔	✔	✔	✔	✔	✔	✔	X	8
13	Ward	Medicine	No	✔	X	✔	✔	✔	X	✔	✔	✔	7
14	Ward	Medicine	Yes	✔	✔	✔	✔	✔	✔	✔	✔	X	8
15	POAC	Surgery	Yes	✔	✔	X	✔	X	✔	✔	X	✔	6
16	POAC	Surgery	No	✔	✔	✔	✔	✔	✔	✔	✔	X	8
17	Ward	Surgery	Yes	✔	✔	✔	✔	✔	X	✔	✔	X	7
18	POAC	Surgery	No	✔	✔	✔	✔	✔	X	✔	✔	X	7
19	Ward	Medicine	Yes	✔	✔	✔	✔	X	✔	✔	✔	X	7
20	POAC	Surgery	Yes	X	X	X	✔	✔	✔	✔	✔	X	5
21	Ward	Medicine	No	✔	✔	✔	✔	✔	✔	✔	X	X	7

Table 2 *Results of the Medical Basic Specialist Trainee Group. Questions 7 and 10 being discarded.*

Participant Number	Q1	Q2	Q3	Q4	Q5	Q6	Q8	Q9	Q11	Result
1	X	✔	X	X	✔	✔	✔	✔	X	5
2	X	X	X	✔	✔	✔	✔	✔	✔	6
3	✔	✔	✔	✔	X	✔	X	✔	✔	6
4	✔	✔	✔	✔	✔	✔	✔	✔	X	8
5	✔	✔	X	✔	✔	X	X	✔	X	5
6	✔	X	✔	✔	X	X	✔	✔	X	5
7	✔	X	✔	✔	✔	✔	✔	✔	X	7
8	X	✔	✔	✔	X	✔	✔	✔	X	6
9	✔	✔	X	✔	✔	✔	✔	✔	X	7
10	✔	X	X	✔	✔	✔	X	✔	X	5
11	✔	✔	✔	✔	✔	✔	✔	✔	X	8
12	✔	✔	✔	✔	X	✔	✔	✔	✔	8
13	X	X	✔	X	✔	✔	✔	✔	X	5
14	✔	✔	✔	✔	✔	✔	✔	✔	✔	9
15	✔	X	✔	✔	✔	✔	✔	✔	✔	8
16	✔	✔	✔	✔	✔	✔	✔	✔	✔	9
17	X	✔	✔	✔	✔	✔	✔	✔	X	7
18	X	X	✔	✔	✔	X	✔	✔	X	5
19	✔	X	✔	✔	✔	✔	✔	X	X	6
20	✔	✔	✔	✔	✔	X	✔	✔	X	7
21	✔	✔	X	✔	✔	✔	✔	✔	✔	8
22	✔	X	✔	✔	✔	✔	✔	✔	✔	8
23	✔	✔	✔	✔	✔	✔	✔	✔	X	8
24	✔	✔	✔	✔	X	✔	✔	✔	X	7
25	✔	X	✔	✔	X	✔	✔	✔	X	6
26	✔	X	✔	✔	✔	✔	✔	✔	X	7
27	✔	✔	✔	✔	✔	✔	✔	✔	X	8
28	✔	X	✔	✔	✔	✔	✔	✔	X	7
29	✔	✔	✔	X	✔	X	✔	✔	X	6
30	✔	X	✔	X	✔	✔	✔	✔	X	6

Forced Vital Capacity (FVC) and the Forced Expiratory Volume in the first second (FEV1) are important parameters that are measured. The FVC is the volume of air forcefully expired up to completion starting from full inspiration. The FEV1 is the volume of expired air in the first second of an FVC manoeuvre.

The patient should be provided with all the relevant information about the test and on what medications

to withhold prior the test. This, together with operator feedback and display of flow on a screen, helps improve patient experience and give more accurate results. 3 key elements required for high quality spirometry are:

- accurate instrumentation;
- acceptable, repeatable measurements by a co-operative patient;
- motivated and trained operator to ensure best performance by patient.

Tests performed while standing yield similar results but FEV1 and FVC may be higher. Patient age, gender, height (in centimetres) and weight (wearing indoor clothes and without shoes) are recorded; these values will provide a predicted value for the FEV1 and FVC, which would then be expressed as a percentage compared to the recorder values.

Before lung function testing, patients should be advised to avoid smoking (from 1 hour before), alcohol/intoxicants (8 hours), vigorous exercise (1 hour) and wearing tight clothing. Bronchodilators should be stopped if the study is performed to diagnose an underlying lung condition. If spirometry is done to assess response to an existing therapeutic regimen, these need not be withheld. The medication, dose and time of last administration should be recorded. Inhaled corticosteroids and leukotriene modifiers need not be withheld.

The largest values of FEV1 and FVC are reported and their ratio used for the FEV1/FVC. Values may not necessarily come from the same manoeuvre. Pre- and post-bronchodilator values are recording using both percentages and absolute values. As mentioned in the results section, the new recommendation by the European Respiratory Society and American Thoracic Society on reversibility of FEV1/FVC, is that there needs to be a 10% change relative to the predicted FEV1/FVC, rather than a 12% change (or 200ml change)[5]. Three acceptable FEV1 and FVC values are usually enough. The manoeuvre can be repeated up to 8 times but this increases the risk of fatigue.

When comparing to the literature, a similar audit was performed by Kamdar et al[6]. The results of the latter indicated a similar trend of results after subjects were assessed with a questionnaire; also improvement of the results were noted after appropriate training in spirometry interpretation, this could be adapted locally (for example, via an online summary on the basic interpretation of spirometry, available easily on the national hospital network) and then performing a re-audit.

Although relatively satisfactory scores were obtained from the two groups in our audit, it is still concerning that a significant number of participants answered basic questions incorrectly; these basic principles being important in the interpretation of a spirometry result, especially in the context of patients having a pre-operative assessment or those who are being investigated with obstructive and/or restrictive lung diseases. It is particularly concerning to note that Question 2, which asks a basic question with regards to the cut-off for an obstructive FEV1/FVC Ratio, was answered incorrectly by 13 participants in the BST group.

It is recommended that basic interpretation skills of spirometry should be part of the post-graduate learning curriculum, either in the form of lectures or tutorials. Another suggestion could be that an information sheet can be present with the spirometry result, which would aid interpretation and direct further investigations or consultation with a respiratory physician. This can also be addressed through implementation of online education resources, guidelines and information material (locally referred to as clinibytes) which is readily available to all doctors locally.

Limitations of this audit included the relatively low number of participants required to achieve statistical significance, as well as a disproportionate number of participants who were willing to participate in each respective group. There was also no formal randomisation with regards to data collection.

CONCLUSION

Further education is required to standardise the knowledge on the basic interpretation of spirometry, in both foundation doctors and medical basic specialist trainees. Interpretation of lung function testing is important as this could affect management, including organising further investigations or identification of important reversible obstructive lung diseases such as Asthma.

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ORIGINAL ARTICLE

Genomic characteristics of meningococcal carriage amongst young adults in Malta

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Background: Invasive meningococcal disease has an incidence of 0.4-3/100,000 in Malta. Meningococcal carriage studies shed light on the epidemiology, transmission, and pathogenesis of invasive meningococcal disease. This research aimed to investigate the prevalence and genomic characteristics of meningococcal carriage in young adults aged 18-24 years attending the only university in Malta.

Methods: Two posterior pharyngeal swabs were taken from 404 university students, and tested for *Neisseria meningitidis* (Nm) by culture and polymerase chain reaction. Isolates were assigned a serogroup and genogroup, and underwent whole genome sequencing to identify sequence type (ST), clonal complex (CC) and Bexsero antigen sequence type (BAST). Diversity amongst carried isolates was assessed using Simpson's Index of Diversity (D).

Results: Twenty-five students (6.2%; 95% C.I. 4-9%) were carriers for Nm While most meningococcal isolates were non-serogroupable (n=14; 66.7%; 95% C.I. 43-85.4%), the predominant genogroup was B (n=9; 36%; 95% C.I. 18–57.5%), followed by Y (n=6; 24%; 95% C.I. 9.4 – 45.1%). Fourteen different ST distributed among 9 CC, and demonstrating 17 different BAST, were identified amongst the carried meningococcal isolates. There was a high degree of BAST diversity (D=0.98). CC53, CC23 and the hyperinvasive CC41/44 accounted for 4 (19%; 95% C.I. 5.4-41.9%), 4 (19%; 95% C.I. 5.4-41.9%) and 3 (14.3%; 95% C.I. 3-36.3%) of isolates respectively.

Conclusions: Our findings demonstrate a wide biodiversity in meningococcal carriage, with CC23, CC41/44 and CC53 important. The introduction of glycoconjugate MenACWY vaccines on the national immunisation schedule could reduce meningococcal carriage and potentially IMD in Malta.

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Invasive meningococcal disease (IMD) is a devastating illness, with a 10% mortality rate, and morbidity in 8-20% that includes sensorineural deafness, limb amputations, and neurological disability.[1] *N. meningitidis* (Nm), the causative agent of IMD, is often carried asyptomatically in the throat, with rates varying from 4.5% in infants to 23.7% in adolescence.[2] The rarity of IMD compared with oropharyngeal meningococcal carriage (OMC), and the much wider genetic diversity amongst carried compared to invasive meningococcal strains, suggests that the Nm life cycle is dependent on transmissibility and carriage, rather than disease.[3] IMD incidence in Malta varied between 0.4-3/100,000 individuals per year from 2013-2017, compared to an overall European incidence of 0.5-0.7/100,000 individuals/year over the same period.[4] Meningococcal vaccination was only introduced on the national immunisation schedule in June, 2020.

We conducted a meningococcal carriage study at the only university in Malta. The aim of the study was to establish carriage rates amongst university students aged between 18 and 24 years, and describe the microbiological and molecular diagnostic profile of both OMC and IMD in Malta. This study is the first to present whole genome sequencing (WGS) data of carried meningococcal isolates in Malta. By characterising circulating carried meningococci in terms of their sequence type (ST), clonal complex (CC), and vaccine antigen sequence types, WGS will provide a detailed analysis of the potential for carried meningococci to cause IMD locally, as well as identify opportunities for vaccination.

MATERIALS AND METHODS

The objective of this study was to assess the point prevalence and describe the epidemiology and genetic characteristics of OMC amongst university students in Malta. The inclusion criteria were: students at the University of Malta (UoM), aged 18-24 years of age. Criteria for exclusion from the study were meningococcal vaccination in the prior 5 years, previous meningococcal disease, active oro/nasopharyngeal infection, and recent antibiotic use (within the previous 2 weeks). A sample size of at least 400 subjects was chosen to allow confidence intervals of 95%, based on an estimated prevalence of meningococcal carriage of 5-10%, an α of 0.05 and a β of 0.80, and a population aged 18-24 years reported in Malta in 2017 of 40,840.[5]

Students were recruited after university lectures. Consenting participants were asked to complete a questionnaire assessing risk factors for OMC. Two cotton-tipped swabs (Cliniswab Sterile Transport Swab, Nuova Aptaca, Canelli, AT, Italy, and ∑-VCM, MWE Medical Wire, Wiltshire, U.K) were then taken simultaneously from the posterior pharynx on each participant. One swab was placed in Amie's transport medium, and the second in Universal Transport Culture Medium (UTCM). Both swabs were transported to the Mater Dei Hospital microbiology laboratory within 4 hours, where the charcoal swab was immediately plated onto Thayer-Martin medium with antimicrobial supplement (vancomycin, colistin, nystatin, trimethroprim lactate) and incubated at 37°C in 5% carbon dioxide. The plates were inspected at 24, 48 and 72 hours: plates with a positive growth were subcultured onto chocolate agar, and underwent a Gram stain to confirm the presence of Gram-negative diplococci. Nm identification was done using Matrix-assisted laser desorption/ionisation-Time-of-Flight mass spectrometry (MALDI-TOF MS) (VITEK MS, bioMerieux, Marcy L'Etoile, France). Identification of capsular serogroup was done by means of slide agglutination with commercial antisera based on whole cell preparations for capsular groups A, B, C, D, W, X, Y, and Z (Remel Agglutinating Sera, *Neissera meningitidis*, ThermoFisher Scientific, Waltham, MA, USA). DNA was extracted from the carried meningococcal isolates using the QIAamp DNA Mini kit (Qiagen, Hilden, Germany) and the QIAcube machine (Qiagen, Hilden, Germany). Genogrouping of the isolates was done by rt-PCR (M-DiaSero, Diagenode diagnostics, Belgium) targeting the *sacB*, $siaD_B$, $siaD_C$, *synG* and *synF* genes for detection of genogroups A, B, C, W, and Y respectively, and performed using the Qiagen Rotor-Gene Q (Qiagen, Hilden, Germany). The DNA extracts were transferred to the Oxford Genomics Centre (OGC) at -70°C, where samples were quantified using the Quant-iT™ PicoGreen ® dsDNA Kits (Invitrogen, ThermoFisher Scientific, Waltham, MA, USA) and a FLUOstar OPTIMA plate scanner (BMG Labtech, Ortenburg, Germany), and libraries constructed using

the NEBNext Ultra DNA Sample Prep Master Mix Kit (NEB, Ipswich, MA, USA). Each library was PCR-enriched using custom dual index primers.[6] After amplification the reactions were purified with Agencourt AMPure beads (Beckman Coulter Life Sciences, Indianapolis, IN, USA). Sequencing was done using an Illumina NovaSeq6000 (Illumina NovaSeq, Illumina Inc., San Diego, CA, USA) as 150bp paired end.

The second oropharyngeal swab from each participant was stored in UTCM (∑-VCM, MWE Medical Wire, Wiltshire, U.K.) at 4°C. The AutoMate Express Instrument (Applied Biosystems, ThermoFisher Scientific, MA, USA) was used to extract 50µL of DNA from 400µL of the UTCM. The DNA extracts were stored at -20°C, and subsequently underwent rt-PCR targeting the *porA* gene for meningococcal detection (BactoReal kit Nm porA gene, Ingenetix, Bioactiva, Austria). Those extracts that were positive on PCR alone underwent subsequent genogrouping by rt-PCR as above (M-DiaSero, Diagenode diagnostics, Belgium).

Data analysis was done using Statistical Package for the Social Sciences® (SPSS). Confidence intervals were calculated using Binomial exact calculation.[7] Simpson's Index (D) was used as indicators of genetic diversity amongst CC and vaccine antigen sequence types, and calculated using the Columbia University Biodiversity Calculator.[8] A D closer to 0 was taken as indicative of increasing diversity, indicating a larger number of unique vaccine antigen sequence types or CC amongst the sequenced meningococcal isolates. Odds ratios for assessing risk factors for OMC were calculated according to Altman, 1991.[9] Post-sequencing analysis was performed using the Bacterial Isolate Genome Sequence Database (BIGSdb), including MLST and vaccine antigen sequence typing for the four-component MenB (Bexsero®, MenB-4C) and Bivalent rLP2086 (Trumenba®, MenB-fHbp) vaccines using BAST.[10] BAST analysis with the BIGSdb was used to estimate vaccine coverage with MenB-4C and MenB-fHbp.[10] Comparisons between the findings of this study and pooled meningococcal isolates from across Europe from a comparable population aged 18-24 years were made by accessing the Bacterial Isolate Genome Sequence Database (BIGSdb).[10]

RESULTS

There were 404 participants in total, aged between 18 and 24 years, with a mean age of 20 years (95% CI 19.9 - 20.2). The M:F ratio was 1:1.2, with 223 (55.2%) female participants. Three hundred and eighty-nine participants were of Maltese nationality (96.3%). Forty-five participants described themselves as smokers (11.1%; 95% CI: 8.2-14.6%). Most participants shared their household with 3 (n=168; 41.6%; 95% CI: 36.7-46.6%) or 4 (n=109; 27%; 95% CI 22.7-31.6%) other people. Assessing the risk factors for meningococcal carriage was not one of the objectives of this study, though a significant association was noted between OMC and attendance to public houses or discotheques at least twice a week (OR=4.62, 95% CI: 1.41-15.15; p=0.01).

Twenty-five (6.2%; 95% CI: 4-9%) participants were found positive for OMC on either *porA* PCR or culture (**Figure 1**). Twenty-one (5.2%; 95% CI: 3.2-7.8%)

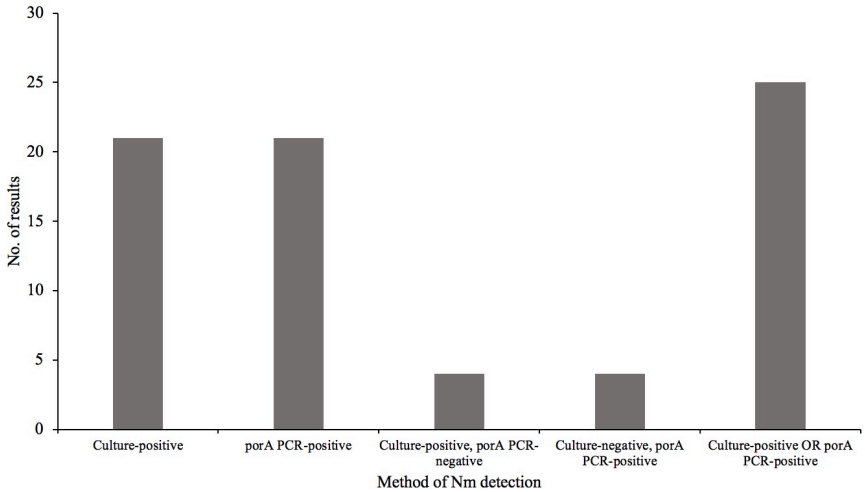


Figure 1 *Number of positive results with each method of Nm detection performed on throat swabs. (PCR: Polymerase Chain Reaction)*

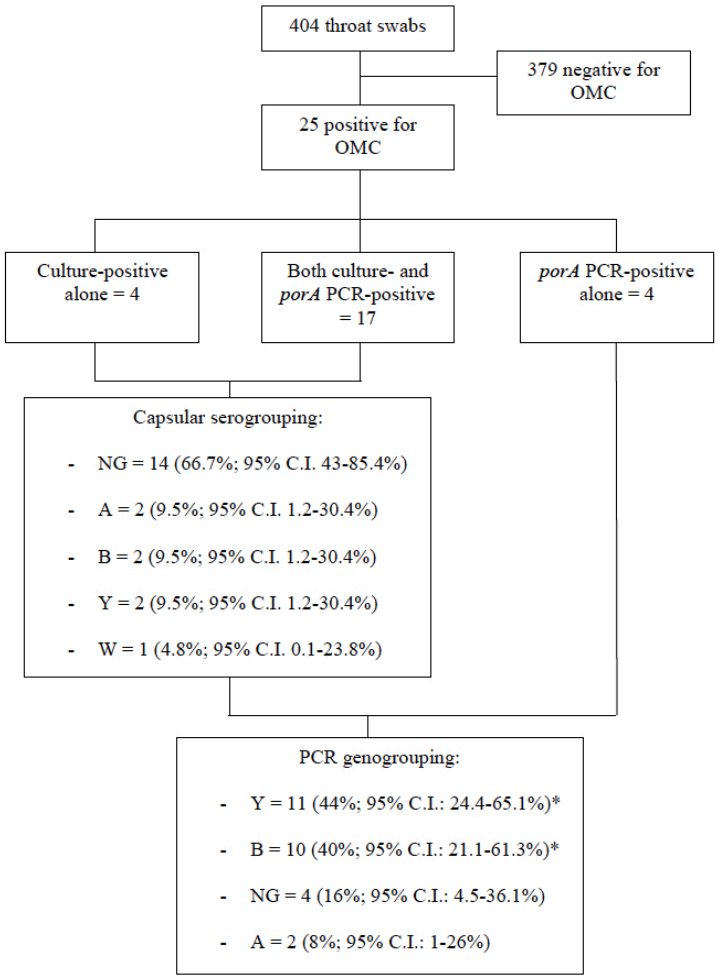


Figure 2 *Capsular serogrouping and PCR genogrouping of carried meningococcal isolates. (OMC = oropharyngeal meningococcal carriage; PCR = polymerase chain reaction; NG = non-groupable) *Two samples were positive for both genogroups B and Y on genogrouping PCR.*

charcoal throat swabs were positive for Nm on culture and MALDI-TOF. Seventeen of these were also positive for Nm on *porA* PCR (81%; 95% C.I.: 58.1-94.6%). Twenty-one samples were positive for Nm on *porA* PCR (5.2%; 95% C.I.: 3.2-7.8%). Therefore, culture successfully identified 4 cases of meningococcal carriage missed on *porA* PCR, while *porA* PCR identified 4 cases of carriage not identified by culture and MALDI-TOF. Of the cultured meningococcal isolates (n=21), most were found to be non-groupable on capsular serogrouping (n=14, 66.7%; 95% CI: 43-85.4%), with genogroups Y (n=11; 44%; 95% CI: 24.4-65.1%) and B (10; 40%; 95% CI: 21.1-61.3%) being the most prevalent of all isolates (culture or PCR) (Figure 2). Two samples were identified as both genogroup B and Y.

On WGS, 6 carriage isolates were found to belong to genogroup B (28.6%; 95% CI: 11.3-52.2%), 5 to genogroup Y (23.8%; 95% CI: 8.2-47.2%), 2 were genogroup E (9.5%; 95% CI: 1.2-30.4%), 1 was genogroup W (4.8%; 95% CI: 0.1-23.8%), 1 non-genogroupable (4.8%; 95% CI 0.1-23.8%), and the remaining 6 possessed the capsule null locus (cnl) (28.6%; 95% CI: 11.3-52.2%). Fourteen different sequence types (ST) were identified amongst the carried meningococcal isolates, with 2 isolates of unidentified ST (Figure 3). The commonest ST was ST-53 (n=4; 19%; 95% CI: 5.4-41.9%), one of 3 STs found to have the capsule-null locus (cnl) on genogrouping,

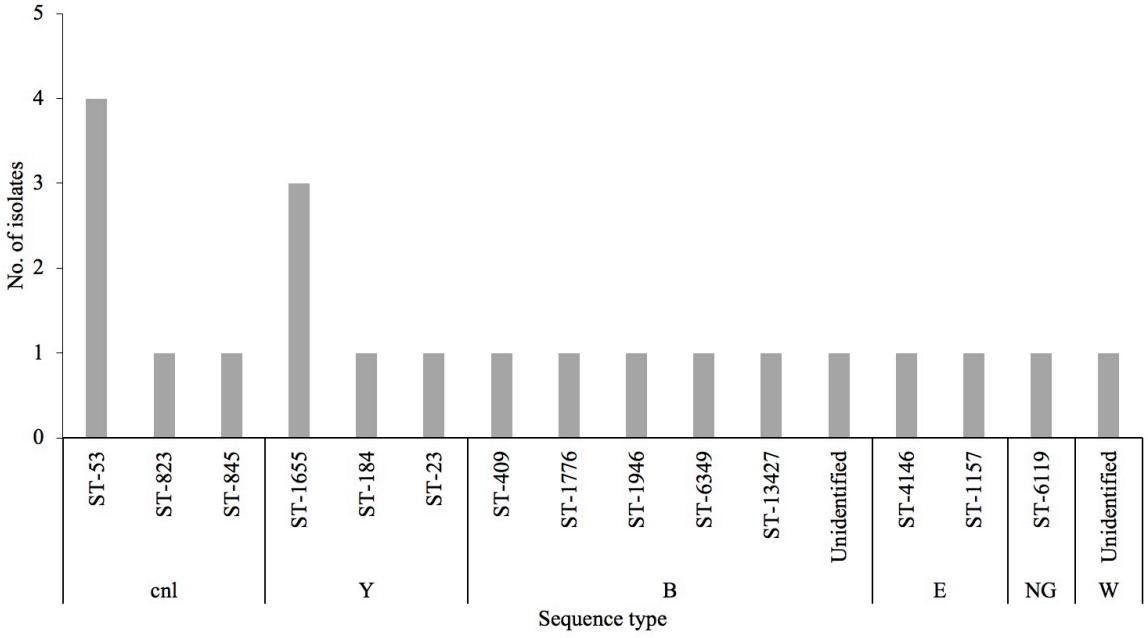


Figure 3 *Distribution of sequence types amongst carrier meningococcal isolates (cnl: capsule null locus, B: genogroup B on WGS, Y: genogroup Y on WGS, E: genogroup E on WGS, NG: non-groupable on WGS, W: genogroup W on WGS).*

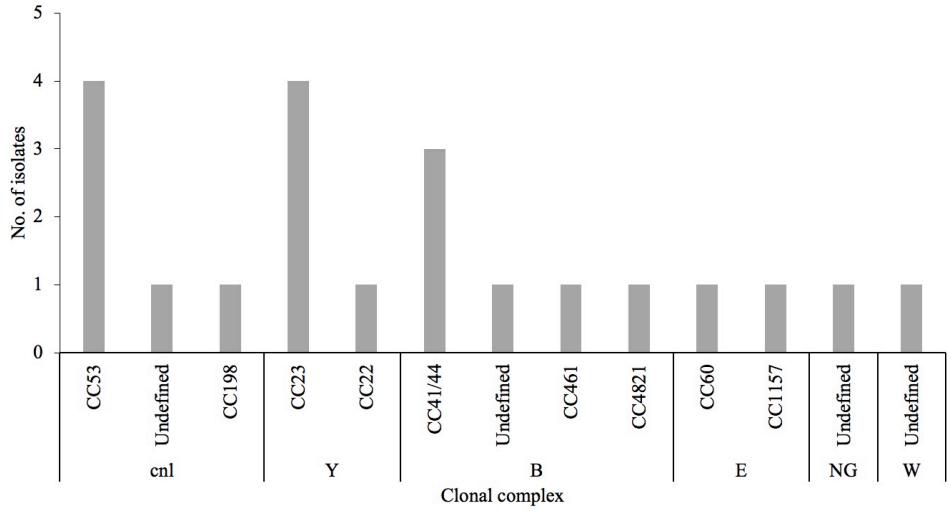


Figure 4 *Distribution of clonal complexes amongst carrier meningococcal isolates (cnl: capsule null locus, B: genogroup B, Y: genogroup Y, E: genogroup E, NG: non-groupable, W: genogroup W; all on WGS).*

followed by the Y genogroup ST-1655 (n=3; 14.3%; 95% CI: 3 – 36.3%). D for the carried isolate ST was found to be 0.053. Four isolates each (19%; 95% CI: 5.4-41.9%) belonged to the ST-23 and ST-53 CCs (Figure 4). Three isolates (14.3%; 95% CI: 3-36.3%) belonged to the hyperinvasive ST-41/44 CC. D for the carried isolate CC was 0.11.

Seventeen different BAST were recorded amongst 20 carried isolates (BAST for 1 isolate remained undefined), with 3 isolates of BAST-22 (15%; 95% CI: 3.2-37.9%), and the remaining isolates each of a different BAST. The ratio of unique BAST to isolates was 1:1.12, and there was a high degree of BAST diversity, with a D of 0.02. Of the 6 genogroup B carried isolates, 2 isolates (33.3%; 95% CI: 4.3-77.7%) showed exact or cross-reactive matches for MenB-4C antigens. On the other hand, all 6 of the genogroup B carried isolates (100%; 95% CI:54.1-100%) showed exact or cross-reactive matches for MenB-fHbp antigens.

Comparing our findings to pooled European isolates available on BIGSdb, serogroup A and non-serogroupable meningococci accounted for significantly greater proportions of carried meningococci locally compared to pooled European isolates (MenA: 9.5% vs 0.5%, p<0.0001; NG: 66.7% vs 42.7%, p=0.03), while serogroup B was significantly underrepresented locally (9.5% vs 34.1%, p=0.018) (Table 1). Furthermore, CC23, CC53, and CC4821 accounted for a significantly higher proportion of Maltese than European carried isolates (5.7% vs 19% [p=0.012], 6.5% vs 19% [p=0.025], 0% vs 4.8% [p<0.0001] respectively) (Table 2).

DISCUSSION

OMC is essential for Nm transmission, influencing IMD epidemiology and the effectiveness of vaccination.[3] We showed Maltese university students aged 18-24 years, an age-group at high risk for OMC, to have a meningococcal carriage rate of 6.2%. The meningococcal carriage rate in the Mediterranean region in a comparable young adult population

Table 1 *Capsular groups of carried meningococci in European participants aged 18-24 years, compared with Maltese carried isolates in a similar sample population*

Capsular group	European carriage (N=979)		Maltese carriage (N=21)		
	n	% (95% CI)	n	% (95% CI)	*p*-value
A	5	0.5 (0.2-1.2)	2	9.5 (1.2-30.4)	***<0.0001***
B	335	34.1 (31.2-37.2)	2	9.5 (1.2-30.4)	***0.018***
C	75	7.6 (6.1-9.5)	0	0 (0-16.1)	0.19
E	35	3.6 (2.5-4.9)	0	0 (0-16.1)	0.38
W	33	3.4 (2.3-4.7)	1	4.8 (0.1-23.8)	0.73
X	14	1.4 (0.8-2.4)	0	0 (0-16.1)	0.59
Y	57	5.8 (4.4-7.5)	2	9.5 (1.2-30.4)	0.48
Z	6	0.6 (0.2-1.3)	0	0 (0-16.1)	0.72
NG	419	42.7 (39.6-45.9)	14	66.7 (43-85.4)	***0.03***

Table 2 *Distribution of clonal complexes amongst carried meningococci in Malta and Europe, in individuals aged 18-24 years (CC = clonal complex)*

	European carriage (N=773)		Maltese carriage (N=21)		
CC	**n**	**% (95% CI)**	**n**	**% (95% CI)**	***p*-value**
32	33	4.3 (3-5.9)	0	0 (0-16.1)	0.33
11	42	5.4 (3.9-7.3)	0	0 (0-16.1)	0.27
41/44	179	23.2 (20.2-26.3)	3	14.3 (3-36.3)	0.34
23	44	5.7 (4.2-7.6)	4	19 (5.4-41.9)	***0.012***
53	50	6.5 (4.8-8.4)	4	19 (5.4-41.9)	***0.025***
461	6	0.9 (0.4-1.8)	1	4.8 (0.1-23.8)	0.08
60	23	3 (1.9-4.4)	1	4.8 (0.1-23.8)	0.64
4821	0	0 (0-0.5)	1	4.8 (0.1-23.8)	***<0.0001***
22	43	5.6 (4.1-7.4)	1	4.8 (0.1-23.8)	0.87
198	39	5 (3.6-6.8)	1	4.8 (0.1-23.8)	0.97
1157	7	0.9 (0.4-1.9)	1	4.8 (0.1-23.8)	0.08
35	41	5.3 (3.8-7.1)	0	0 (0-16.1)	0.28
269	20	2.6 (1.6-4)	0	0 (0-16.1)	0.45
162	20	2.6 (1.6-4)	0	0 (0-16.1)	0.45
92	29	3.8 (2.5-5.3)	0	0 (0-16.1)	0.36
106	22	2.8 (1.8-4.3)	0	0 (0-16.1)	0.44
254	21	2.7 (1.7-4.1)	0	0 (0-16.1)	0.45

ranged from 2% to 18%.[11-18] However, meningococcal carriage is dynamic, changing over time and in different regions, with a systematic review showing meningococcal carriage in university students to range from 1.5% to 71.1%.[19] Therefore, the Maltese carriage rate appears comparable with that in other regions, and most likely subject to similar temporal fluctuations. Frequent attendance to discotheques or public houses was found to be a significant risk factor for carriage (OR: 4.62, 95% CI: 1.41-15.15; p=0.01) similar to what has been reported in other studies.[20]

The Nm polysaccharide capsule protects meningococci from phagocytosis, opsonisation and complement-dependent bacteriolysis, and is the major antigen to generate bactericidal antibodies after infection or vaccination (with the exception of serogroup B vaccines).[21] The role of the capsule in invasion explains why invasive isolates are rarely acapsular, and therefore non-serogroupable, compared with carriage isolates, of which 66.7% were non-serogroupable in this study.[22] There are various mechanisms behind failure of capsule expression: six isolates in this study carried the *cnl* locus, without potential for capsule expression, while 9 isolates (64.3%) were non-serogroupable despite an intact *cps* locus, suggesting down-regulation of capsule expression during carriage.[23,24]

The proportion of non-serogroupable and serogroup A carriage isolates were significantly higher in Malta compared to pooled European carried isolates from participants 18-24 years (9.5% vs 0.5%, p<0.0001), while that of serogroup B was lower (9.5% vs 34.1%, p=0.018). Aside from geographical variation, these differences may relate to discrepancies in the year of analysis: local isolates were collected in 2018, at a time of low rates of IMD-MenB, while the European isolates date back to the 1970s. The proportion of serogroup B in carriage in more recent Mediterranean studies has varied between 9.4% in Turkey and 43% in Italy.[11,13] MenA is very rarely

identified in Europe, accounting for a limited proportion of IMD and OMC in the Eastern European region and Asia, and is more important in Africa: its presence in our sample may therefore relate to Malta's proximity to the African continent, and thus the influence of migrants and tourists from this and other regions where MenA carriage has been documented.[25-28] It is notable, however, that both MenA isolates detected in this study contained the cnl locus, raising doubts about the reliability of their assigned serogroup.

The carriage isolates were mostly genogroup B (36%) and Y (24%), with 6 isolates bearing the cnl locus (24%). These results align themselves well with similar recent findings from countries in the Mediterranean region, where non-genogroupable and genogroup B isolates were found to predominate, followed by genogroup Y.[14,15,18,29] Genogroup E, while rarely responsible for IMD in Europe or Malta, accounted for a similar proportion of local carriage to that in Spain, Greece and Italy.[14,18,29,30] The absence of genogroup C in carriage locally contrasts with the proportion of Maltese IMD attributed to this serogroup.[30] While the low rate of MenC carriage in other European studies suggests that the sample size of our study might not have been large enough to detect MenC carriage, the prevalence of MenC amongst invasive isolates in Malta possibly implies a preference for invasion rather than carriage, which may be short-lived and thus missed by a point prevalence study.[30] Since conjugate meningococcal C vaccination was not part of a national schedule locally at the time of this research, this finding is unlikely to be a result of herd immunity, which has reduced MenC carriage overseas. [11-13,15,18,29,31]

While carried isolates tend to be clonally heterogeneous, most IMD is caused by a limited number of hyperinvasive CCs, including CC5, CC41/44, CC32, CC11, CC269, and CC8.[32,33] This is reflected in this study: CC distribution was wide amongst carried isolates, with no single predominant CC or ST. Hyperinvasive CCs are rarely found in carriage, exhibiting evolutionary advantages in transmission and disease, but not in carriage, where non-invasive meningococci predominate.[34] Only 3 carriage isolates belonged to a hyperinvasive CC (CC41/44). CC23 and CC53 were important in carriage, accounting for 19% of isolates each, and accounted for a significantly higher proportion of Maltese compared with European carriage (CC23: 19% vs 5.7%, p=0.012; CC53: 19% vs 6.5%, p=0.025). CC53 is also prevalent in carriage in Italy, a country which neighbours Malta and is both a source of tourists and a popular tourist destination.[12,18] CC4821 accounted for one carriage isolate locally, and was not otherwise present amongst European carriage isolates (4.8% vs 0%, p<0.0001). CC4821 first emerged in IMD-MenC outbreaks in China from 2003-2005.[35] It has since been responsible for cases of IMD-MenB and IMD-MenC, as well as carriage, across the world.[36] CC198, accounting for 4.8% of our carriage isolates, is reported amongst cases of IMD with cnl meningococci.[37]

Pharyngeal carriage provides a stable ecological niche for Nm, and a fertile platform for high rates of genetic recombination.[33] This is reflected in the broad diversity of both CC and BAST in carriage isolates in this study: carriage allows Nm to sustain a high rate of horizontal genetic transfer that leads to greater genomic diversity. This could confer advantages vis-a-vis invasiveness and transmissibility, allowing new hyperinvasive clones, with novel antigenic combinations, to emerge, with the potential for

SUMMARY BOX

What is already known about this subject:

- Oropharyngeal carriage of *N. meningitidis* (Nm), the causative agent of invasive meningococcal disease, occurs frequently in adolescents.
- Meningococcal vaccination programs are most effective when they impact both meningococcal disease and carriage.
- Invasive meningoxcoccal disease occurs with an incidence of 0.4-3/100,000 in Malta.

What are the new findings:

- University students in Malta carried oropharyngeal meningococci at a rate of 6.2%.
- The majority of carriage isolates did not express a capsule.
- Genogroups B, Y and W, and CC23, CC41/44 and CC53 predominated in carriage.
- Carried meningococci exhibited wide genomic biodiversity.

epidemic or hyperepidemic spread.[38] Genomic diversity amongst carried meningococci may undermine the potential for newer protein-based vaccines to generate herd protection by reducing meningococcal carriage, a phenomenon seen with conjugate meningococcal vaccines.[39] The high BAST diversity in our carriage sample reinforces such concerns locally. BAST allows a prediction of MenB vaccination efficacy by identifying the genes for the antigens that influence their immunogenicity: a high degree of BAST diversity and a low prevalence of BAST-1, the BAST profile that matches MenB-4C antigens precisely, suggest poor coverage of prevalent meningococcal MenB-4C antigens.[40] A difference in estimated coverage of MenB carriage isolates with MenB-4C and MenB-fHbp was seen in this study, showing potentially superior MenB-fHbp coverage (OR 13.6 [95% CI: 3.1-59.8], p=0.0006). However, no conclusions can be drawn from this, as neither of the currently available MenB vaccine formulations has been shown to impact carriage.[41-43]

Meningococcal conjugate vaccines have been shown to reduce carriage, leading to reduced acquisition and IMD incidence.[44] The presence of capsule-expressing MenY and MenW suggests that MenACWY might have a more significant impact on local meningococcal carriage than MenC vaccination alone. Furthermore, the acapsular MenY isolates found in carriage have the potential to express a capsule through phase variation. With MenC vaccination alone, MenY and MenW may persist in carriage and be transmitted to cause disease in susceptible individuals.[38]

These results will shed no light on vaccine effectiveness or cost-effectiveness in this population without vaccine introduction. The recruitment of subjects excluded those with factors that may have influenced meningococcal carriage, though may be subject to recall bias. The discrepancies between the genogrouping results obtained by rt-PCR compared with WGS suggest a number of inaccuracies in the former, partly explained by the fact that the rt-PCR genogrouping kit used in this research (M-DiaSero, Diagenode diagnostics, Belgium) did not include primers to detect genogroup E (2 isolates). As only 25 participants were found to be positive for meningococcal carriage, sub-analyses of the positive isolates is limited. Lastly, coverage estimates were based entirely on genomic data, while protein expression was not measured: certain isolates may possess antigenic genes without expressing antigenic protein in vivo, potentially leading to overestimates of the predicted Meningococcal B vaccine coverage.

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CONCLUSION

Students aged 18-24 years at the only university in Malta carried oropharyngeal meningococci at a rate of 6.2%. Although the majority of carried isolates did not express a capsule, genes coding for the MenB, MenY and MenW capsules were identified, proving circulation of these genogroups within the community. The wide genomic biodiversity shown in carriage strengthens its role as a platform for genetic exchange and meningococcal adaptation, with potential to generate new hyperinvasive lineages. The introduction of glycoconjugate MenACWY vaccines on the national immunisation schedule could reduce meningococcal carriage, and potential IMD in Malta.

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ORIGINAL ARTICLE

Audit on Risk Stratification and Follow-up of Pulmonary Embolism as per 2019 ESC guidelines

Matthew Grech, Giulia Stringaro, Etienne Ceci Bonello, Emma Mifsud, Brendan Caruana Montaldo

Background: Pulmonary embolism is a common and potentially life-threatening disease. The aim of this audit is to determine whether the necessary investigations for risk stratification were taken, including troponin and N-terminal pro B-type natriuretic peptide to assess for heart strain and whether appropriate follow-up was provided with echocardiography and outpatients appointment as per 2019 ESC guidelines on pulmonary embolism.

Method: Retrospective review of computed tomography pulmonary angiographies (CTPA) performed from 1st July 2022 to 31st December 2022 at Mater Dei Hospital and Gozo General Hospital. Documentation on investigations carried out and appropriate follow-up were collected from Picture Archiving and Communication System (PACS) and iSOFT Clinical Manager.

Results: A total of 1682 CTPAs were carried out over the investigated period, out of which 12.8% (n=216) had a PE. Only 34.7% had troponin levels checked and 25.5% had NT-pro-BNP levels checked. Right heart strain was reported on CTPA in 13.9% of patients with a PE. In these patients, testing of cardiac biomarkers was higher with 70.0% of them having at least one cardiac biomarker tested. 30.1% of patients with PE had an echocardiogram done, most of which (73.8%) were as outpatients. More patients (56.7%) had an echo done when right heart strain was documented. Only 42.6% of patients had appropriate outpatients follow-up within 6 months of discharge.

Conclusion: A structured management plan in the form of local guidelines is necessary which will aid the emergency physician and also the internal medical clinician to treat and follow-up appropriately.

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Pulmonary embolism is a common and potentially life-threatening disease which involves thrombosis of the pulmonary vasculature, most commonly arising from a deep vein thrombosis of the lower limbs or pelvis. Treatment delays in this condition can be fatal hence the importance of timely diagnosis and management cannot be overemphasised.

Apart from clinical evaluation of the patient, the 2019 ESC guidelines for the diagnosis and management of pulmonary embolism (PE) recommend diagnostic investigations for confirmation of PE diagnosis and risk stratification, some of which assess the effect of the embolism on the heart.[1]

Evidence of heart strain may be assessed with blood tests, namely troponin and N-terminal pro B-type natriuretic peptide (NT-pro-BNP), and also with imaging, namely a computed tomography pulmonary angiography (CTPA).[2] According to the 2019 ESC guidelines, echocardiography is not required as a routine diagnostic work-up for patients with haemodynamically stable PE. However, in patients with evidence of heart strain as established by elevated cardiac biomarkers or on CTPA, echocardiography can be an additional aid to identify, and risk stratify the degree of heart strain.[3] The investigation findings together with clinical assessment of the patient will assist the clinician to categorise the patient into high, intermediate, or low risk, hence influencing management, including the consideration of thrombolysis apart from anticoagulation.

Furthermore, guidelines also suggest that patients with PE should have a follow-up at 3-6 months to assess for dyspnoea and/or functional limitation. Therefore, risk stratification and appropriate follow up is necessary to determine the long-term management of such patients.

MATERIALS AND METHODS

The aim of this audit is to determine whether the necessary investigations for risk stratification and appropriate follow-up was provided to patients who developed pulmonary embolism as per 2019 ESC guidelines.

Retrospective review of CTPAs performed from 1st July 2022 to 31st December 2022 at Mater Dei Hospital and Gozo General Hospital was done (n=1682), out of which 12.8% (n=216) were found to have PE. This information was collected using the Picture Archiving and Communication System (PACS). Documentation of right heart strain on CTPA reports was also noted via PACS.

Information with regards to patient demographics such as age, gender, investigations done, accident and emergency notes, and follow-up appointments was then gathered from the Mater Dei Hospital software including iSoft Clinical Manager and Electronic Case Summary (ECS). Permission for this audit was obtained from the Mater Dei Hospital (MDH) CEO, Legal office and Data Protection office. No ethical approval was required for this audit.

RESULTS

All CTPAs done between 1st July 2022 and 31st December 2022 were evaluated (n=1682), of which 12.8% were found to have a PE (n=216). Out of these, 52.8% (n=114) were female and 47.2% (n=102) were male. The mean age was 66 years.

Out of 216 positive CTPAs, 25.0% (n=54) had active malignancy, 14.8% (n=32) had a past history of venous thromboembolism, including DVT (n=15; 46.9%), PE (n=13; 40.6%) or both (n=4; 12.5%) and 21.8% (n=47) had a surgical procedure in the previous month, the majority of which were a total knee replacement (n=13; 27.7%).

From the CTPAs that were evaluated, 50.5% (n=109) were done at the accident and emergency department or within 24 hours of admission to hospital. 47.7% were done whilst the patient was an inpatient. In 4 patients (1.9%), the patient was not admitted after being found to have a PE. Out of those who had a PE-positive CTPA whilst they were an inpatient, the average length of stay prior to the CTPA was 7.9 days.

As recommended by the 2019 ESC guidelines, 2 cardiac biomarkers were taken into consideration in this audit: troponin and NT-pro-BNP. For these results to be included in the audit, the cardiac biomarkers must have been taken on the day of the CTPA or up to 24 hours after. Additionally, in cases where a second repeat troponin was taken, only the second troponin was taken into consideration.

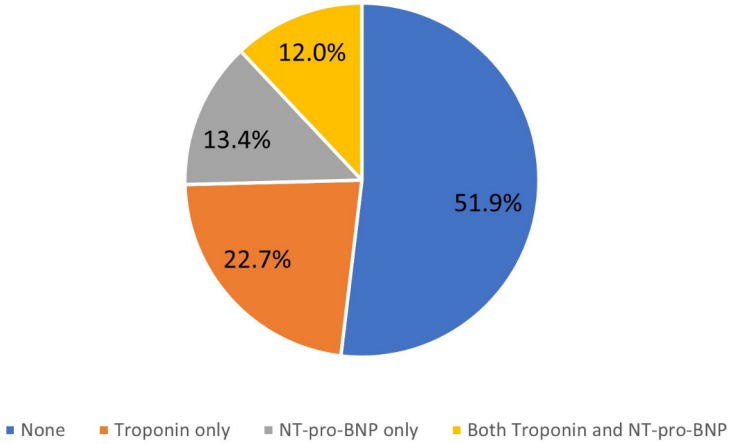


Figure 1 *Figure showing which cardiac biomarker has been tested in those who were found to have a PE on CTPA*

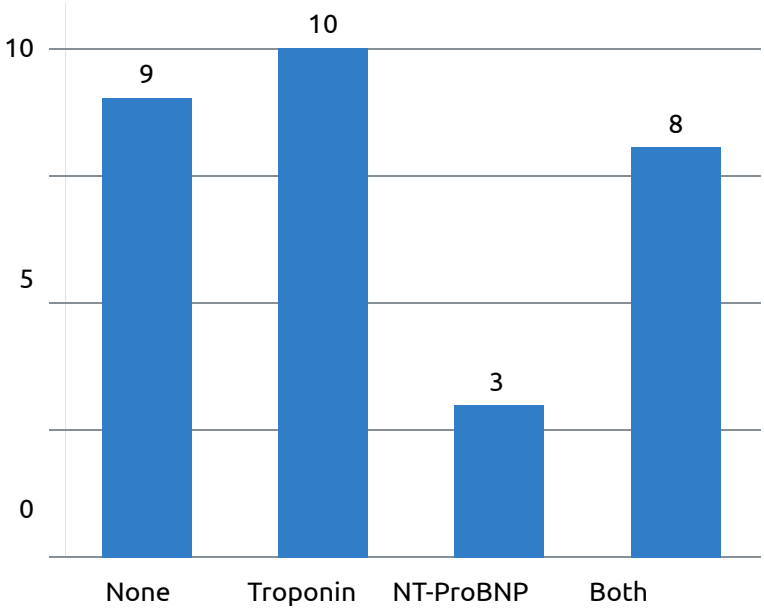


Figure 2 *Figure showing which cardiac biomarkers were tested in patients who had PE with right heart strain*

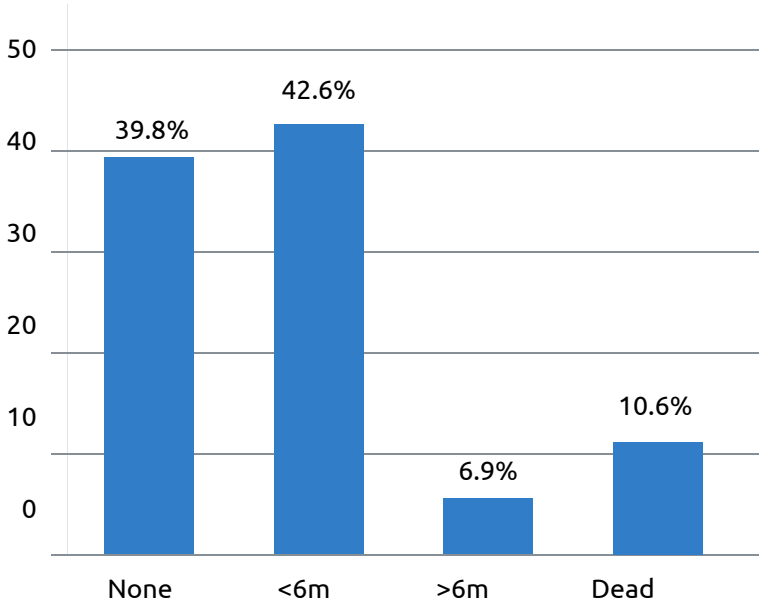


Figure 3 *Figure showing follow-up percentages after PE diagnosis*

75 patients (34.7%) from the total cohort had their troponin level checked of which 39 (52.0%) were elevated (>/= 15pg/ml). With regards to NT-pro-BNP, 55 patients (25.5%) had their levels checked, out of which 44 (80.0%) were elevated (>/=126pg/ml). Out of the 216 patients who were found to have a PE, only 26 (12.0%) had both troponin and NT-pro-BNP levels checked on the day of the CTPA or up to 24 hours after (**Figure 1**).

With regards to identification of right heart strain, 30 patients (13.9%) were noted to have right heart strain on CTPA. From these, 21 (70.0%) had at least one cardiac biomarker checked (**Figure 2**).

Out of the 30 patients who had evidence of right heart strain on CTPA, only 1 patient underwent thrombolysis – the patient had a normal troponin (6) but an elevated NT-pro-BNP (933). From the 186 patients who had a PE but did not have evidence of right heart strain, only 1 patient underwent thrombolysis who was found to have an elevated troponin (110) but a normal NT-pro-BNP (72).

An echocardiogram was carried out in 30.1% (total = 65) of the patients who were found to have a PE either at A&E, as an inpatient or as an out-patient after the patient was discharged. From these, a total of 48 (73.8%) had the echo done as an out-patients while the rest (26.2%) were done at A&E or as an inpatient. Out of those patients who had documented right heart strain on CTPA, 17 patients (56.7%) had an echo done.

The 2019 ESC guidelines recommend a follow-up up within 6 months of PE diagnosis. With regards to this audit, follow-up with the caring medical firm or haematology are being taken as adequate follow ups. From the 216 patients who were found to have a PE, 23 (10.6%) of them had passed away during their admission thus a follow-up could not be provided to them. Out of the remaining 193 patients, only 47.7% (92 patients) were provided with a follow-up within 6 months of diagnosis (**Figure 3**).

DISCUSSION

Results of this audit show that risk stratification in PE using the cardiac biomarkers troponin and NT-pro-BNP was sub-optimal with more than half (51.9%) of

SUMMARY BOX

- We recommend testing of cardiac biomarkers, including troponin and NT-pro-BNP to assess for degree of heart strain in patients with PE.
- In those patients with elevated cardiac biomarkers or where heart strain is documented on CTPA, they should undergo echocardiography.
- When deciding treatment of PE such as with thrombolysis, the investigations should be taken into consideration with the clinical picture of the patient.
- Follow-up of patients with PE is necessary, ideally within 6 months to identify patients with persistent symptoms, those who are developing CTED/CTEPH and those who might benefit from pulmonary rehabilitation.

patients having neither of these investigations taken on the day that the CTPA was done and up to 24 hours after. In patients who had a PE, these cardiac biomarkers were elevated in the majority of patients, with troponin being elevated in 52.0% and NT-pro-BNP elevated in 80.0%, thus indicating possible cardiac compromise which can lead to an increased risk of clinical deterioration.[4]

Another important identifier for risk stratification is the presence or absence of right heart strain. Most of the time this was documented in the CTPA report. In those patients with PE and documented right heart strain, it was noted that the rate of testing for cardiac biomarkers was greater than in the general cohort with 70% of patients with right heart strain having had at least one cardiac biomarker tested.

The same trend can be seen with regards to echocardiography. In the general cohort, only 30.1% of the patients had an echo done during their stay in hospital or as an outpatients, however a greater proportion of patients (56.7%) had an echo when right heart strain was documented in the CTPA report. This indicates that documentation of right heart strain on CTPA makes clinicians more likely to assess for cardiac compromise using blood tests and imaging.

The majority of echocardiograms (73.8%) were done as an outpatients after the patient was discharged, with the echo being booked upon discharge from their inpatient stay or booked when the patient is seen at outpatients.

The 2019 ESC guidelines on PE suggest that patients who are found to have a PE should have an outpatients follow-up within 3-6 months of diagnosis.[5] In this audit, follow-up was only considered if it was with the caring medical firm or with haematology. Unfortunately, when excluding the patients who had passed away during their admission, less than half (47.7%) of patients had a follow-up that was within the 6-month time frame from when they were diagnosed.

The first limitation of the study is that it is retrospective. Data was collected from MDH software, and documentation may not have always been available, complete or documented appropriately. Another important limitation was that other co-morbidities that the patient suffered from, such as ischaemic heart disease and congestive heart failure were not taken into consideration, which may have independently contributed to an elevated troponin or NT-pro-BNP. Another limitation was that clinical assessment status was not taken into consideration in this audit, which in clinical practice is essential to guide the correct individualised management plan especially when considering thrombolysis.

CONCLUSION

Based on the results of this audit, in patients found to have a PE, appropriate risk stratification is vital, as well as thorough investigations using cardiac biomarkers and echocardiography when a CTPA shows heart strain. Likewise, it is important to follow-up such patients to assess for symptoms and functional limitation. The lack of follow up can result in morbidity and mortality especially from conditions such as chronic thromboembolic disease/pulmonary hypertension (CTED/CTEPH), which might result from PE, and which will go undiagnosed without appropriate follow up. Our recommendations are a structured management plan especially in the form of local guidelines, which will aid the emergency physician and also the internal medical clinician to treat and follow-up appropriately.

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ORIGINAL ARTICLE

Unveiling Noradrenaline: Insights into Cardiac Autonomic Neuropathy in a Type 1 Diabetic Animal Model

Shamala Devi Subramaniam, Nurulaidah Esa, Noorkardiffa Syawalina Omar, Razif Abas

Background: The global surge in Type 1 Diabetes Mellitus (T1DM) cases across age groups has raised concerns about associated cardiovascular risks, warranting a deeper understanding of its complications. Diabetic Cardiovascular Autonomic Neuropathy (DCAN), characterized by cardiovascular system irregularities in diabetic patients, remains inadequately explored, particularly in T1DM. This study aimed to establish a connection between T1DM and the onset of DCAN in an animal model, focusing on the role of noradrenaline as a potential marker.

Methods: Male Wistar rats were randomly divided into Control and T1DM model groups. The diabetic group received intraperitoneal Streptozotocin injections and remained untreated until DCAN developed. Blood glucose levels and body weight were measured weekly. Vital signs monitoring, and noradrenaline measurements were conducted at specific intervals using non-invasive techniques and ELISA kits to assess the rats' cardiovascular parameters and noradrenaline levels throughout the study period.

Results: Subsequent analysis revealed distinct alterations in blood glucose, body weight, blood pressure, and heart rate in the diabetic group, mirroring clinical symptoms observed in diabetes. Notably, a significant rise in serum noradrenaline levels at week 14 suggested the emergence of DCAN.

Discussion: Findings demonstrate a parallel between T1DM and the development of DCAN, echoing observations in human diabetic patients. Importantly, this study highlights the potential of noradrenaline as an early indicator of DCAN onset. Despite limitations in sample size and duration, these groundbreaking insights pave the way for refining diagnostic strategies and developing targeted therapies for this debilitating complication in diabetic patients.

Conclusion: This investigation offers critical insights into the pathophysiology of DCAN in T1DM, emphasizing the urgent need for early detection and management strategies to mitigate associated cardiovascular risks in diabetic individuals.

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While type 1 diabetes primarily affects children, a growing number of adults either developed the condition during childhood or acquired it later in life. Consequently, a substantial portion of individuals living with type 1 diabetes face an increased risk of cardiovascular diseases as they reach adulthood. Notably, this disease's incidence is rising globally, exhibiting considerable variations across different nations and regions, possibly due to diverse environmental factors. Factors like perinatal events and hygiene in early life have been suggested as influential, while studies on body weight's link to type 1 diabetes have yielded mixed results.[1]

Surprisingly, some research indicates a rise in type 1 diabetes cases among young individuals but not in adults, yet the reasons behind this discrepancy remain inadequately explored compared to type 2 diabetes. Recent global statistics reveal that approximately 60% of the estimated 9 million people with type 1 diabetes in 2017 were over 40 years old, an age bracket predisposed to prevalent cardiovascular risk factors and emerging cardiovascular complications. Intriguingly, despite high-income countries hosting only 17% of the world's population, they accounted for 49% of new Type 1 diabetes cases.[2]

Diabetic cardiovascular autonomic neuropathy (DCAN) refers to the impairment of the autonomic regulation of the cardiovascular system in individuals with diabetes, excluding other potential causes.[3] It arises from damage to the nerve fibres that control the heart and blood vessels, leading to irregularities in cardiovascular functioning. DCAN is a prevalent chronic complication in both type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM), linked to elevated morbidity and mortality rates in diabetic patients.[3] While the confirmed prevalence of DCAN in unselected individuals with T1DM and T2DM is approximately 20%, higher figures of up to 65% have been reported with advancing age and longer duration of diabetes. The development of diabetic CAN involves multiple factors, including heightened mitochondria production of free radicals due to hyperglycaemia-induced oxidative/nitrosative stress.[3]

Catecholamines encompass a cluster of hormones and neurotransmitters, comprising dopamine, norepinephrine (noradrenaline), and epinephrine (adrenaline). These substances serve pivotal roles in the body's reaction to stress and contribute significantly to diverse physiological functions. Studies have examined the appearance rate and clearance of noradrenaline in idiopathic autonomic neuropathy and diabetic cardiovascular autonomic neuropathy (DCAN).[4] Assessing catecholamines in diabetes has revealed generally diminished responses to postural changes, like exercise, hypoglycaemia, and cardiovascular autonomic reflex tests in humans. To comprehensively gauge whole-body sympathetic activity and relevant physiological aspects (such as heart rate, blood pressure, cardiac output, and hormonal and metabolic events), it is imperative to measure plasma concentrations of noradrenaline, adrenaline, and plasma 3,4-dihydroxyphenylglycol (DHPG).[4]

Given the scarcity of studies concentrating on T1DM, our aim is to establish a connection between this disease and the emergence of diabetic cardiovascular autonomic neuropathy in an animal model, with a specific focus on noradrenaline. Consequently, these groundbreaking findings are expected to significantly aid future research aimed at diagnosing DCAN more effectively.

MATERIALS AND METHODS

Male Wistar rats weighing about 180-200g were purchased from the Comparative Medicine and Technology Unit (COMeT). The animal operation was reviewed and approved by the Institutional Animal Care and Use Committee at Universiti Putra Malaysia (IACUC). The animals were placed in the Animal Behaviour Laboratory, Human Anatomy Department, in clean cages. The room was kept at a regulated temperature and humidity, with a 12-hour light/dark cycle. All animals were given two weeks to adapt to their new surroundings before the studies began. They were housed in wooden cages in pairs and were fed freely with pellets.[5]

After adaptation, the rats were randomly divided into a Control group (n=10) and a Type 1 Diabetic model (DM) group (n=30). The DM groups were injected intraperitoneally with a high dose of Streptozotocin (STZ) 50mg/kg.[6] Subsequently, while continuing to be

fed rat pellets and housed in pairs with wood shavings, all diabetic rats remained untreated until the establishment of DCAN.

Prior to the morning blood glucose measurement using a glucometer (Accu-check Guide Meter), the rats were fasted starting at 2000H the night before.[6] Blood glucose levels were measured for Week 0, Week 3, Week 18, Week 19, and Week 20. Blood was collected from a rat's tail that had been sterilised with an alcohol swab, and the last 3 mm was snipped off before the blood glucose test paper was inserted into the glucometer.[6] Body weight from week 0 to week 20 using an (Adam Weighing Balance) was recorded weekly.[5]

Non-invasive echocardiography and blood pressure monitoring were conducted using a tail cuff and a CODA High Throughput Monitor (Kent Scientific, Torrington, CT). Measurements were taken at Week 0, 3, 10, 18, 19, and 20, with readings measured over 3 cycles for each rat. Any undesirable or erratic recordings—such as data falsely generated by the system—were excluded from the analysis. The parameters collected included systolic (SBP), diastolic (DBP), mean (M) blood pressure, and heart rate (HR) for all the rats.

Disposable test tubes were used to collect whole blood samples for noradrenaline measurement according to the kit's instructions (Elabscience Biotechnology, China) at Week 10 and Week 14. The ELISA kit uses the competitive ELISA principle. The ELISA plate provided in this kit had been pre-coated with Noradrenaline/Norepinephrine (NA/NE). During the reaction, NA in samples or standard competed with a fixed amount on the solid-phase supporter for sites on the Biotinylated Detection Ab Specific to NA. Excess conjugate and unbound samples were washed from the plate, and Avidin conjugated to Horseradish Peroxidase (HRP) was added to the microplate well and incubated. Then, the TMB substrate solution was added to each well. The enzyme-substrate reaction was terminated by adding a stop solution, and the colour change was measured spectrophotometrically at a wavelength of 450nm. The concentration of NA in the samples was then determined by comparing the OD of the samples to the standard curve.[7]

RESULTS

The Effects of STZ on Fasting Blood Glucose Levels

Hyperglycaemia had been confirmed by Week 2 following the STZ injection. As anticipated, the negative control group (n=6) exhibited normal glucose levels compared to the experimental group (n=30) (Figure 1). Glucose levels were significantly elevated from Week 3 through to Week 18. Moreover, diabetic rats demonstrated increased urine production, leading to frequency and polyuria until Week 18.

The Effects of DM on Body Weight

In Week 0, the body weight of all the rats was recorded before the acclimatization week. The negative control group (n=6) and experimental groups (n=30) were monitored weekly for body weight changes. Despite the administration of STZ in Week 1, there was no significant increase in body weight observed for either the negative control or experimental group from Week 1 to Week 2 (Figure 2). However, starting from Week 3 until Week 18, the body weight significantly decreased in the experimental group compared to the negative control, while the body weight consistently increased in the negative control group (statistical significance not shown).

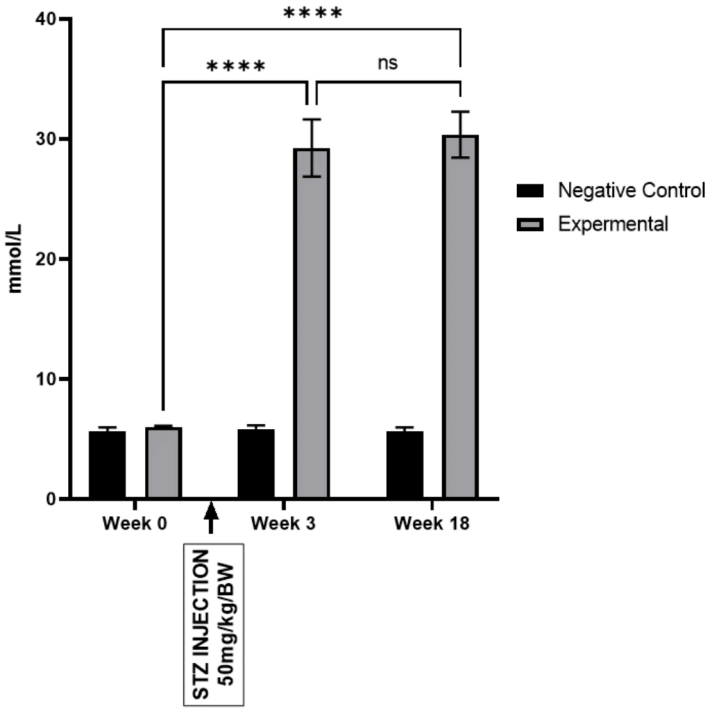


Figure 1 *STZ administration took place in Week 1. Blood glucose levels displayed a significant increase from Week 3 onwards and remained elevated until Week 18 (ns= no significant, ****p<0.0001).*

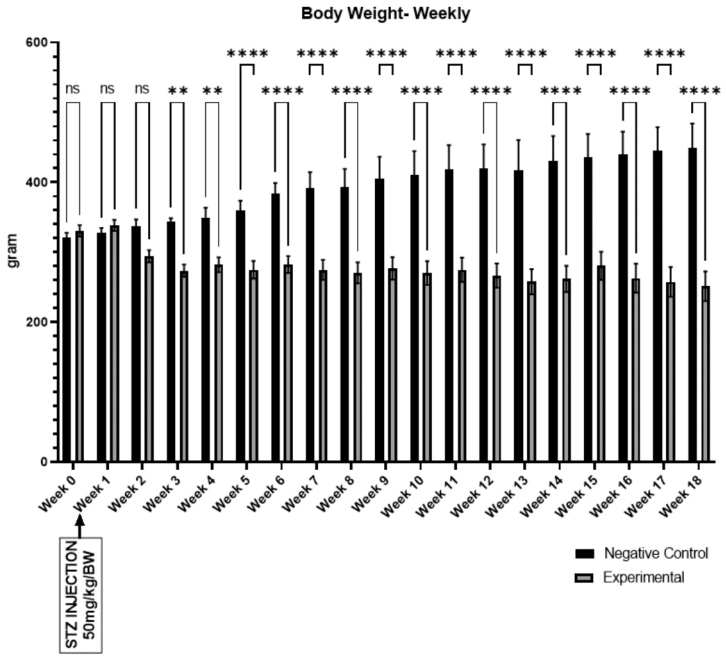


Figure 2 *The graph bars depicted a decreasing trend in body weight within the experimental group compared to the negative control group, commencing from Week 3 and persisting until Week 18 (ns= no significant, **p<0.01, ****p<0.0001).*

The Effects of DM on Blood Pressure (systolic, diastolic and mean) and Heart Rate

The effects of blood pressure and heart rate following STZ injection in Week 1 are illustrated in **Figure 3**. In the negative control group (n = 6), there were no significant changes observed in any parameters: systolic, diastolic, mean blood pressure, or heart rate (data not shown). However, both systolic and mean blood pressure exhibited a notable increase in the experimental group (n=30), commencing at Week 3 and nearly plateauing after Week 10. Additionally, the experimental group displayed bradycardia in heart rate, starting from Week 3 and maintaining a nearly constant level thereafter. No significant changes in diastolic pressure were observed within the experimental group.

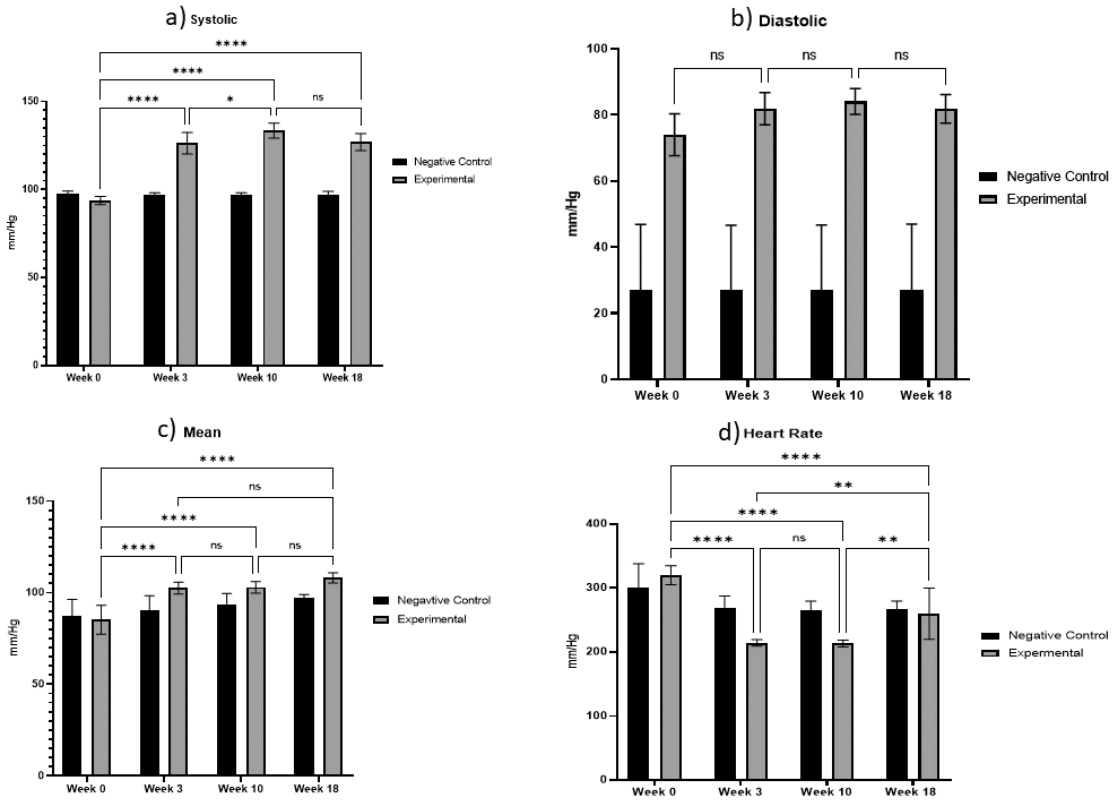


Figure 3 *The blood pressure measurements, including (a) systolic, (b) diastolic, (c) mean, and (d) heart rate, were compared to analyse the trends in both the experimental and negative control groups. Significant increases were observed in systolic and mean blood pressure within the experimental group starting from Week 3. There were no notable changes detected in diastolic blood pressure. Additionally, heart rate demonstrated a significant increase from Week 3 onwards in the experimental group (ns = no significant, **p<0.01, ****p<0.0001).including (a) systolic, (b) diastolic, (c) mean, and (d) heart rate, were compared to analyse the trends in both the experimental and negative control groups. Significant increases were observed in systolic and mean blood pressure within the experimental group starting from Week 3. There were no notable changes detected in diastolic blood pressure. Additionally, heart rate demonstrated a significant increase from Week 3 onwards in the experimental group (ns = no significant, **p<0.01, ****p<0.0001).*

The Effects of DM on Serum Noradrenaline (NA)

The ELISA results revealed a substantial rise in serum NA levels, notably higher in the experimental group (n=30) compared to the negative control group (n=6) by week 14 (Figure 4). Although there was an increased concentration of NA in Week 10, it did not reach statistical significance. The findings at week 14 suggest that the animals were already exhibiting signs of Diabetic Cardiac Autonomic Neuropathy.

DISCUSSION

The current study aimed to establish a connection between T1DM and the emergence of DCAN in an animal model, with a specific focus on noradrenaline. The investigation commenced by confirming the relevance of the study through an introductory exploration of the increasing incidence of T1DM globally. Notably, this condition, primarily considered a paediatric concern, has witnessed a surge in adulthood cases, exposing a larger population to cardiovascular risks as they age.[1]

Our findings corroborate the well-established prevalence of DCAN in both T1DM and T2DM. The confirmed prevalence of DCAN in unselected individuals with T1DM and T2DM approximates 20%, with figures soaring to 65% with prolonged diabetes duration and advancing age. This aligns with previous clinical observations of heightened morbidity and mortality rates in diabetic patients due to DCAN, underscoring the critical need for early diagnosis and effective management strategies.[3]

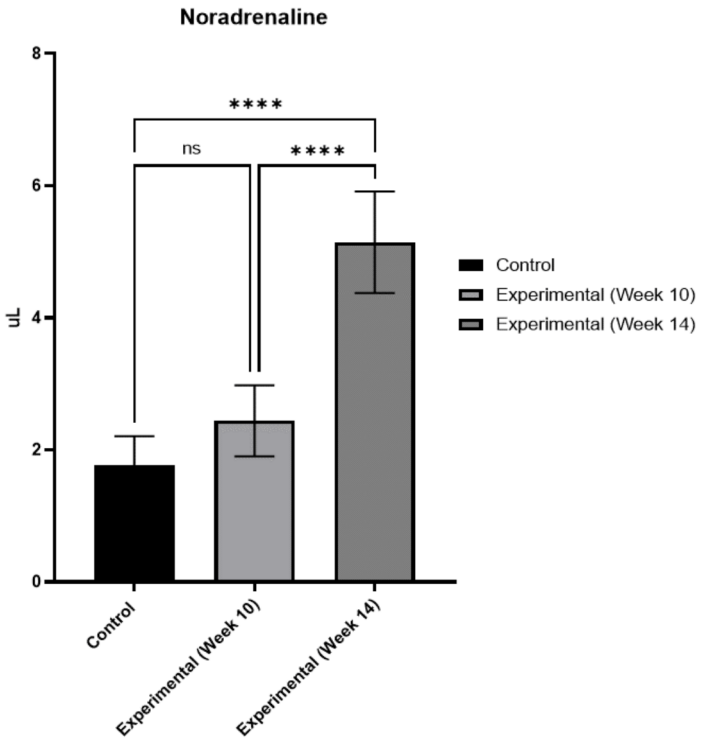


Figure 4 *The serum NA levels showed a significant increase by Week 14, indicating the establishment of DCAN. No additional measurements were conducted at Week 18 due to the limited availability of chemicals (ns= no significant, ****p<0.0001).*

SUMMARY BOX

- T1DM, once considered a childhood disease primarily, is increasingly diagnosed in adults, thereby elevating long-term cardiovascular risk. Its global incidence varies markedly across regions, possibly due to environmental influences such as perinatal factors and early-life hygiene, while associations with body weight remain inconclusive.
- The rise in cases is more pronounced among youth than adults, with high-income countries contributing disproportionately to new diagnoses.
- DCAN, prevalent in longstanding diabetes, damages autonomic nerve fibres that regulate cardiac function.
- Altered catecholamine activity, including noradrenaline, further disrupts cardiovascular responses, contributing to impaired autonomic regulation and increased morbidity over time.

The study effectively induced hyperglycaemia in the experimental group following STZ administration, simulating the diabetic condition, as evidenced by elevated blood glucose levels.[8] Concurrently, these diabetic rats displayed characteristic clinical signs such as increased urine production and reduced body weight, consistent with diabetic symptoms observed in animal models and clinical settings.[9]

An intriguing discovery from our investigation relates to the alterations observed in blood pressure and heart rate. The experimental group exhibited a notable increase in systolic and mean blood pressure levels, alongside a consistent bradycardic trend in heart rate. These alterations, akin to findings in diabetic patients, might denote an early onset of cardiovascular autonomic dysfunction in the animal model.[4] Notably, the absence of significant changes

in diastolic pressure aligns with some clinical observations in diabetic individuals, highlighting the complex nature of cardiovascular responses in DCAN.

Crucially, our study aimed to shed light on the role of NA as a potential marker for DCAN. The ELISA results revealed a substantial rise in serum NA levels, particularly pronounced at week 14, suggesting the onset or progression of DCAN. While the increase in NA concentration at week 10 did not reach statistical significance, the marked elevation at week 14 strongly indicates the establishment of DCAN in the animal model.

Our study has some limitations, including a relatively small sample size and the lack of long-term observations beyond week 18. Additionally, further exploration of other catecholamines, alongside additional biomarkers related to DCAN, could provide a more comprehensive understanding of autonomic neuropathy in diabetes.

ACKNOWLEDGEMENT

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CONCLUSION

In conclusion, our pioneering investigation successfully established a link between T1DM and the emergence of DCAN in an animal model, emphasising the potential utility of noradrenaline as an early marker for this condition. These findings offer valuable insights into the pathophysiology of DCAN and pave the way for future research aimed at refining diagnostic approaches and developing targeted therapies for this debilitating diabetic complication.

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ORIGINAL ARTICLE

Micro-organisms play roles in colorectal cancer, is it true?

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Background: Normal gut bacterial floras play a direct or indirect role in the initiation and progression of tumour by inducing chronic inflammation and producing toxins and metabolites. However, data on the types of bacteria that are significant in the sigmoid colon and rectal tumour are still lacking. This study determined the types of bacteria that are associated with the risk of developing sigmoid colon and rectal cancer.

Methods: This case control study involved patients with bowel symptoms and had been subjected to elective colonoscopy procedure from December 2013 to September 2014. Standard bowel preparation was carried out two days prior to colonoscopy either at home or in ward. Biopsy samples were sent to microbiology laboratory for cultivation. The mean of bacterial colonies in the mucosa of sigmoid colon tumour and rectal tumour were compared with the mucosa of normal sigmoid colon and rectum, respectively.

Results: For normal colonoscopy results, 66 biopsies were taken from bowel mucosa (33 at sigmoid colon and 33 at rectum). Eight patients had sigmoid colon tumour and 25 patients had rectal tumour. The results showed that *Clostridium subterminale*, *Finegoldia magna*, *Enterococcus avium*, *Eggerthella lenta*, *Parvimonas micra*, *Clostridium bifermentans*, beta haemolytic streptococcus, *Streptococcus mutans* and *Morganella morganii* were only present in sigmoid colon tumour and were significantly associated with sigmoid colon cancer ($p = 0.041$). More than 15 types of bacteria were isolated in the rectal tumour compared to the normal rectal mucosa, however, only *Proteus mirabilis* ($p = 0.040$) and *Morganella morganii* ($p = 0.046$) were significant in promoting rectal cancer.

Conclusion: *Clostridium subterminale*, *Finegoldia magna*, *Enterococcus avium*, *Eggerthella lenta*, *Parvimonas micra*, *Clostridium bifermentans*, beta haemolytic streptococcus, *Streptococcus mutans* and *Morganella morganii* were associated with the risk of developing sigmoid colon cancer while *Proteus mirabilis* and *Morganella morganii* were associated with rectal cancer.

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Worldwide, colorectal cancer contributed for the third most common cause of cancer-related deaths.[1] The highest rates of colorectal cancer have been reported in developed countries, as compared to less develop ones.[2] In Malaysia, the first report in the National Cancer Registry which covers period from 1st January 2002 to 31st December 2002 showed a total of 26,089 cancers diagnosed among all residents in Peninsular Malaysia, with 11,815 involving males and 14,274 females.[3] Chinese ethnicity had the highest incidence followed by Indians and Malays.[3] The differences in incidence between these populations bring the possibility of genetic factors contribution in the aetiology of colorectal cancer.[4,5] A previous study showed that incidence of rectal cancer in gender are age related.[6] A study conducted by Hospital Pakar Universiti Sains Malaysia (HPUSM) in 2010 observed a lower overall 5-years survival rate for colorectal patient in HPUSM as compared to developed countries, such as Australia.[7]

The large intestine is the site most heavily colonised by microorganisms in the gatrointestinal tract. This vast number of microorganism collection that lives in coexistence with their hosts has been referred to as the microbiota, microflora or normal flora.[8] Colonisation of the GI tract occurs during the first two years of life and became stable with average composition throughout the adulthood life.[9] Recently, the concept that normal bacterial flora is essential for development of inflammation induced carcinoma has emerged from studies of well-known colonic bacterial floras.[10,11]

The relationship between various environmental factors and cancer of the colon has received much attention since epidemiological studies correlated the incidence of the disease with the degree of economic development. In conjunction with food, the colonic bacteria play an important role in promoting carcinogenesis of the large bowel by inducing chronic bowel inflammation and alteration of metabolites product.[12,13] Therefore, identifying the gut bacterial species involved could be beneficial in understanding their mechanism of action and in preventing the onset of tumour or controlling their advancement.

In 1970s, a study on association between gut floras and colorectal carcinoma was conducted. However, due to technical difficulties in characterizing the faecal microflora, it was not completed until 1995. Stool from three different parts of the world with different diet intake were studied and the results showed different in diet intake will carries different risk of developing colonic cancer.[14]

This study was conducted to identify types of bacteria present in both the mucosa of normal sigmoid colon and rectum and types of bacteria that are significant in the sigmoid colon and rectal tumour.

METHODS

Study design and setting

This is a case control study of patients with bowel symptoms and had been subjected to elective colonoscopy procedure due to variety of bowel symptoms at the Hospital Pakar Universiti Sains Malaysia (HPUSM), Kubang Kerian, Kelantan, Malaysia. The study was conducted in the Endoscopy Unit, Department of Surgery and the Microbiology Laboratory of HPUSM. HPUSM is an academic, tertiary care center, and one of the largest hospitals with colorectal unit in Kelantan.

Participants

All patients who presented with bowel symptoms and had been subjected to elective colonoscopy procedure due to variety of bowel symptoms at the HPUSM between December 2013 and September 2014 were recruited. All patients with age more than 18 years old and above, and patients with any medical illness whose diagnoses are confirmed were included.

Patients whose colonoscopy revealed a mass in the rectum or sigmoid colon, but whose histopathological examination (HPE) indicated only inflammatory changes, were excluded from the study. Additionally, patients with a mass or tumour at a site other than the rectum or sigmoid colon were not included. Written informed consent was obtained from all participating patients.

For the reference population, we selected patients who had normal colonoscopy findings, with no pathology identified serving as control patients. The source population, on the other hand, consisted of patients with positive results for a tumour in either the sigmoid colon or the rectum.

Sample size for this study was calculated using the PS: Power and Sample Size Calculation Version 3.0.12

with the significance level (alpha) 0.05, and the power of study (1 - β) of 80% based on the parameter estimate (true probability of exposure among cases = 0.48) obtained from Moore and Moore.[14] The final targeted sample size was determined by considering 10% drop-out rate. The estimated sample size for this study was 66 samples (33 patients for control patients and 33 patients with tumour).

Colonoscopy and biopsy samples collection

Standard bowel preparation was carried out two days prior to colonoscopy either at home or in ward. This ensured that all patients followed the same dietary regimen for at least three days prior to the procedure: a low-residual diet for one day, only clear fluids for one day, and administration of a laxative (oral fleet) one day before the procedure. Additionally, patients were kept nil by mouth for six hours before colonoscopy.

Colonoscopy was performed by experienced endoscopists according to the current practice at the Endoscopy Unit, HPUSM using Olympus system. For patients with normal colonoscopy findings, indicating the absence of any visible pathological changes, we obtained biopsy specimens from two specific sites of the bowel mucosa: the sigmoid colon and the rectum. These locations were selected due to their representativeness and their common use in the evaluation of colonic microbiota in healthy individuals.

In cases where the colonoscopy yielded positive results, indicating the presence of tumours, biopsy samples were taken directly from the tumour sites. This targeted approach was designed to explore the potential correlation between specific microbial populations and tumourigenesis, in line with our hypothesis that certain organisms may contribute to tumour occurrence and exhibit higher concentrations at the site of pathology.

All biopsies were taken using the biopsy forceps (alligator jaw) with diameter of 2.8 mm. These biopsies were sent to the Microbiology Laboratory, *HPUSM* for cultivation.

Bacterial counts in biopsy samples

For cultivation (i.e., on either aerobic blood agar, anaerobic blood agar, Mc-Conkey agar), five quadrant streak method was used. Growths were inspected and followed by purification to enable identification procedure (gram stain). Subsequently, identification using Biomerium Vitek 2 systems took place. The test reactions from Vitek machine were transmitted to optical system for interpretation of test reactions using different wavelengths in visible spectrum.

After calculations of raw data had been completely performed, test reaction results would appeared as "+", "–", "(–)" or "(+)". Reactions that appear in parentheses are indicative of weak reactions that are too close to test threshold. If a unique identification pattern was not recognized, a list of possible organisms was given, or the strain was determined to be outside database scope.

Ethical approval

An ethical clearance was obtained from the Human Research Ethics Committee, Universiti Sains Malaysia (USM) in Kelantan, Malaysia (No. USM/JEPeM/274.3.(6)).

Data analysis

Descriptive statistics were used to summarise the demographics and clinical characteristics of subjects. Numerical data were presented as means (standard deviation, SD). Categorical data were presented as frequency (percentage). The mean of bacterial colonies present in the mucosa of sigmoid colon tumour and rectal tumour were compared with the mucosa of normal sigmoid colon and rectum, respectively using the independent samples t-tests. Statistical analysis was carried out using IBM SPSS Statistics for Windows ver. 22.0 (IBM Corp., Armonk, NY, USA). The limit of significance was set at 0.05.

RESULTS

Patient demographics and clinical characteristics

A total of 66 patients were recruited in this study after completed the colonoscopy procedure. Thirty-three patients were selected after the colonoscopy showed normal findings (no pathological causes of the symptoms) and another 33 patients were selected after the colonoscopy showed either sigmoid or rectal growth or mass. Overall, the

youngest patient who underwent the colonoscopy was 23 years old and the oldest patient was 88 years old with the mean age of 57.3 years and standard deviation of 14 years.

Majority of the patients were Malay (95%) and only 5% were Chinese. This is consistent with demographic data of Kelantan, Malaysia population where more than 90% of populations are Malay. More male patients presented with the bowel symptoms (n = 39) and 19 patients having positive results of tumour. Out of 27 female patients, 14 patients turn out to have malignancy (Table 1).

Bacterial colonies present in the mucosa of normal sigmoid colon and rectum

Supplemental Figure 1 shows mean of bacterial colonies present in the mucosa of normal sigmoid colon and rectum. About 40 types of bacteria were isolated from both normal sigmoid colon and rectum, and almost all bacteria were anaerobic. The commonest bacteria that were isolated in normal sigmoid colon include *Escherichia coli* (*E. coli*), *Klebsiella pneumonia*, *Collinsella aerofaciens*, *Bacteriodes caccae* and *Fusobacterium mortiferum*. Meanwhile, the commonest bacteria that were isolated in the mucosa of normal rectum were *E. coli*, *Prevotella ovalis*, *Streptococcus sanguinis*, *Bacteroides caccae*, *Bacteroides thetaiotaomicron* and *Enterococcus cloacae*.

Table 1 *Demographic data of the two groups (control patients, n = 33 and patients with tumour, n = 33).*

	Control	Patients with tumour
n	**33**	**33**
Mean age, years (SD)	52.58 (13.75)	61.94 (12.72)
Age range, years	23 - 81	26 - 88
Male, n (%)	20 (60.6)	19 (57.6)
Symptoms of presentation, n (%)		
Abdominal pain	8 (24.2)	15 (45.5)
Abdominal distension	3 (9.1)	8 (24.2)
Vomiting	1 (3.0)	9 (27.3)
Per-rectal bleeding	16 (48.5)	22 (66.7)
Altered bowel habit	23 (69.9)	27 (81.8)
Tenesmus	8 (24.2)	24 (72.7)
Mucus	8 (24.2)	22 (66.7)
Constitutional symptoms	9 (27.3)	21 (63.6)

There were a few types of bacteria that were isolated only in the normal sigmoid colon or rectum. The bacteria that were identified only in the sigmoid colon were *Bifidobacterium* species, *Plesiomonas shigellosis*, *Leuconostoc pseudomesenteroides* and *Comamonas testosteroni*. Types of bacteria that were isolated only from normal rectum include *Staphylococcus hominis*, *Eggerthella lenta*, *Clostridium subterminale*, *Enterococcus hirae*, *Lactobacillus* species, *Peptoniphilus asaccharolyticus* and *Streptococcus sanguinis*.

This result also showed that there was no significant difference in terms of types of bacteria that were present in the mucosa of both normal sigmoid colon and rectum. *Prevotella intermedia*, *Aeromonas hydrophila*, *Streptococcus salivarus*, *Streptococcus parasanguinis*, *Proteus vulgaris*, *Actinomyces naeslundii*, *Fusobacterium varium*, *Enterococcus raffinosus* and *Bacteriodes stercoris* were present in equal number in both normal sigmoid colon and rectum.

Other bacteria appeared to be more in rectum than sigmoid colon. These include non-fermentative *E. coli*, *Klebsiella pneumonia*, *E. coli*, *Streptococcus gallolyticus*, *Streptococcus agalactiae*, *Enterococcus faecium*, *Streptococcus mitis*, *Prevotella oralis*, *Fusobacterium nucleatum*, *Bacteroides thetaiotaomicron*, *Enterococcus cloacae*, *Parabacteroides distasonis* and *Burkhoderia cepacia*. In overall however, bacterial colonisation or community appeared to be more in rectum than sigmoid colon.

Comparison of bacterial colonies present in the mucosa of sigmoid colon tumour and normal sigmoid colon

Supplemental Figure 2 shows the mean of bacterial colonies present in the mucosa of sigmoid colon tumour and normal sigmoid colon. From the result, there were certain bacterial species that were present only in sigmoid colon tumour or normal sigmoid colon. *Clostridium subterminale*, *Finegoldia magna*, *Eggerthella lenta*, *Parvimonas micra*, *Streptococcus mutans*, *Morganella morganii*, *Clostridium bifermentans*, *Enterococcus avium* and

beta haemolytic streptococcus were present only in sigmoid colon tumour.

There were several bacteria which appeared to be more predominant in sigmoid colon tumour compared to normal sigmoid colon. These bacteria include *Enterococcus gallinarum*, *Streptococcus mitis*, *Prevotella intermedia*, *Enterococcus faecalis*, *Bacteriodes fragilis*, non-fermentative *E. coli*, *Bifidobacterium* species and *Proteus mirabilis*.

Aeromonas hydrophila, *Klebsiella pneumonia*, *E. coli* and *Collinsella aeroficiens* were present in both mucosa of sigmoid colon but more dominant in normal sigmoid colon. The rest of the bacteria were only present in the mucosa of normal sigmoid colon.

Table 2 shows a comparison of the mean of bacterial colonies present in the normal sigmoid colon and sigmoid colon tumour. The results showed that *Clostridium subterminale*, *Finegoldia magna*, *Enterococcus avium*, *Eggerthella lenta*, *Parvimonas micra*, *Clostridium bifermentans*, beta haemolytic streptococcus, *Streptococcus mutans* and *Morganella morganii* were only present in sigmoid colon tumour and were significantly associated with sigmoid colon cancer (p = 0.041).

Comparison of bacterial colonies present in the mucosa of rectal tumour and normal rectum

Supplemental Figure 3 shows the mean of bacterial colonies that have been identified in the mucosa of rectal tumour in comparison with normal rectum. Almost all bacteria were present in both mucosa of rectum but more predominant in normal rectum. However, there are a few species which highly colonised rectal tumour. These include *Comamonas testosteroni*, *Staphylococcus hominis*, *Streptococcus parasanguinis*, *Enterococcus gallinarum*, *Streptoccocus mitis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Enterobacter* species, *Streptococcus dysgalactiae*, *Prevotella intermedia*, *Morganella morganii*, *Actinomyces meyeri*, *Enterococcus faecalis*, *Clostridium bifermentans*, *Enterococcus avium*, *Bifidobacterium* species, *Clostridium perfringens*, beta-haemolytic streptococcus, *Aeromonas sobria* and *Proteus mirabilis*.

Table 3 shows a comparison of the mean of bacterial colonies present in the normal rectum and rectal tumour. Even though, more than 15 types of bacteria

Table 2 *Comparison of the mean of bacterial colonies present in the mucosa of sigmoid colon cancer and normal sigmoid colon*

Bacteria	Sigmoid colon	Mean (SD)	t-statistic	*p*-value*
Clostridium subterminale	Normal	0.00 (0.000)	-2.118	***0.041***
	Tumour	0.63 (1.768)		
Finegoldia magna	Normal	0.00 (0.000)	-2.118	***0.041***
	Tumour	0.63 (1.768)		
Enterococcus avium	Normal	0.00 (0.000)	-2.118	***0.041***
	Tumour	0.63 (1.768)		
Eggerthella lenta	Normal	0.00 (0.000)	-2.118	***0.041***
	Tumour	0.38 (1.061)		
Parvimonas micra	Normal	0.00 (0.000)	-2.118	***0.041***
	Tumour	0.38 (1.061)		
Clostridium bifermentans	Normal	0.00 (0.000)	-2.118	***0.041***
	Tumour	0.38 (1.061)		
beta-hemolytic streptococcus	Normal	0.00 (0.000)	-2.118	***0.041***
	Tumour	0.38 (1.061)		
Streptococcus mutans	Normal	0.00 (0.000)	-2.118	***0.041***
	Tumour	0.25 (0.707)		
Morganella morganii	Normal	0.00 (0.000)	-2.118	***0.041***
	Tumour	0.13 (0.354)		

**The independent samples t-test was applied.*

Table 3 *Comparison of the mean of bacterial colonies present in the mucosa of rectal cancer and normal rectum*

Bacteria	Rectum	Mean (SD)	t-statistic	*p*-value*
Proteus mirabilis	Normal	0.18 (0.635)	-2.102	0.040
	Tumour	0.80 (1.528)		
Morganella morganii	Normal	0.00 (0.000)	-2.038	0.046
	Tumour	0.28 (0.792)		

**The independent samples t-test was applied.*

were isolated more in the rectal tumour in comparison with the normal rectum, only two bacteria were significant in promoting rectal cancer which were *Proteus mirabilis* (p = 0.040) and *Morganella morganii* (p = 0.046).

DISCUSSION

The usage of bowel cleanser might have changed the composition of bacterial populations[15,16] or may not have any effects[17], and bacteria which still present are termed mucosal-associated bacteria.[18-20] In addition to that, different bacteria location may have different bacteria amount, i.e., between bacteria in faeces and mucosa of large bowel.[19]

From the results, we can see that *E. coli, Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* were present in mucosa of both normal sigmoid colon and rectum with the highest frequency of *E. coli*. Among all aerobic gram-negative bacilli (AGNB), *E. coli* is the only bacteria that reside in gut of healthy people. It has been postulated that AGNB receptor was expressed on healthy mucosa lining of bowel but are covered by protective fibronectin layer.[21] This postulation maybe true as in this study, we can see that even though biopsy was taken from mucosa of normal bowel, patient might have an underlying acute or chronic illness as presented with bowel symptoms which render them for colonoscopy procedure.

From this study, almost all bacteria isolated from sigmoid colon and rectum were anaerobes such as *Bacteroides,* anaerobic Streptococcus and *Enterococcus* species. These show that Asians still harbour anaerobic bacteria as the commonest commensal flora compared to Western populations[22,23] and this may result from dietary intake and lifestyle of Asians.[24]

Previous study found anaerobic bacteria to be predominant in human colonic wall[25,26] with the commonest bacteria present in rectal population.[27,28] From the current study, more bacteria populations are found in rectum compared to sigmoid colon mucosa with several types of bacteria only present in rectum. These findings are supported by past researches[19,29,30], however there are also researches that contradict these findings.[31] This research show that rectal bacterial populations were more common than sigmoid colon and this can be postulated that it may be a mixture of both bacteria from faecal remnant inside the rectum and also mucosa-associated bacteria. Previous study had highlighted the association of *Streptococcus bovis* with an increased risk of developing colorectal cancer with 94% of *S. gallolyticus* subspecies *gallolyticus* (biotype 1) bacteraemia have been shown to be associated with colorectal cancer while 18% was associated with *S. bovis* biotype 2.[32,33]

A large variety of plant derived substances are present in human diet such as lignans and flavonoids which belong to polyphenols group. Few species of bacteria have been identified which can catalyse conversion of isoflavonoids and ligands into active metabolites which includes *Bacteroides*, *Clostridium*, *Eggerthella*, *Eubacterium* and *Ruminococcus*[34]. This finding in reverse is contradicting with our study in which, we found out that nine types of bacteria were only present in sigmoid colon tumour compared to normal sigmoid colon mucosa and was highly significant in causing colorectal cancer. These include *Clostridium subterminale*, *Finegoldia magna*, *Enterococcus avium*, *Eggerthella lenta*, *Parvimonas micra*, *Clostridium bifermentans*, beta haemolytic streptococcus, *Streptococcus mutans* and *Morganella morganii*. Meanwhile, *Enterococcus gallinarum*, *Streptococcus mitis*, *Prevotella intermedia*, *Enterococcus faecalis*, *Bacteriodes fragilis*, non-fermentative *E. coli, Bifidobacterium* species and *Proteus mirabilis* were present in both groups but more predominant in sigmoid colon tumour. Based on this result, we can postulate that even though dietary substances may have a chemo protective effect, there are still more metabolites produced by gut bacteria which can induced the carcinogenesis process.

Bile acids are the end products of cholesterol metabolism. Cholic acid and chenodeoxycholic acid which constitute primary bile acids will be dehydroxylated and converted into secondary bile acids, deoxycholic acid and lithocolic acid in large bowel by colonic bacteria.[35] Few bacteria which have the ability to conjugate bile acid include *Bacteriodes* species, *Veillonella* species, *Clostridium* species, *Staphylococcus* aureus, *Streptococcus faecalis* and *Bifidobacteria*. These bacteria with different degrees of conjugation ability, through cholate-7-dehyroxylase enzyme, will breakdown 7-OH group from cholic acid thus producing deoxycholate.[36,37]

Few studies have been reported regarding association of bile acids with risk of developing rectal cancer in which the cytotoxic metabolites lead to induction of colorectal cancer.[38] With this finding, our study has been supported with *Clostridium* species have been isolated and significantly associated with sigmoid colon tumour.

Compared to sigmoid colon cancer, only two types of bacteria have been identified to be significantly associated with rectal cancer which were *Proteus mirabilis* and *Morganella morganii*. Even though no direct association between *Proteus mirabilis* and human colorectal cancer has been published, an animal study conducted found that *Proteus mirabilis* and *Klebsiella pneumoniae* were present in *T-bet*$^{-/-}$ x *Rag2*$^{-/-}$ulcerative colitis (TRUC) mice and was also found in faeces of the TRUC mice compared to faeces of normal mice. Our concern is on inflammatory bowel disease as one of the risk factors of developing colorectal cancer and with this data, we can postulate that *Proteus mirabilis* and *Klebsiella pneumonia* appeared to be more predominant in mucosa of inflammation bowel and indirectly associated with colorectal cancer.[39]

Morganella morgani has been identified as one of Sulfate-reducing bacteria (SRB) in which it conducted sulphate reduction process thus producing a great quantity of sulphide to obtain energy. This process in turn would produce the end metabolites, hydrogen sulphide which is a cytotoxic compound that will inhibit oxidation of butyrate. Butyrate is the main energy supplier for colonocytes, and this inhibition will impair intestinal epithelial cell functions that will lead to cell death and finally chronic inflammation.[40] This fact showed *Morganella morganii* also inhibit colonic mucosa of inflammatory bowel which in turn predisposed to development of colorectal cancer.

There were certain limitations within this current study. Firstly, as this study was only comparing the type of bacteria in sigmoid colon and rectal mucosa, the difference of bacterial colonies diversity was not well seen especially in the sigmoid colon tumour compared to rectal tumour biopsy samples. This is because only eight samples of sigmoid colon tumour were collected in this study compared to 25 samples of rectal tumour. This study also only focused on the left side of the large bowel which most probably have the similar types of bacterial residing the mucosa and only involved samples from the normal mucosa in comparison with tumour tissues. While we implemented standard bowel preparation protocols for all participants to minimize dietary confounding, other potential confounders such as medication use and comorbidities were not comprehensively adjusted for. Future research should aim to incorporate more rigorous adjustments for these factors to better isolate the relationship between gut microbiota and colorectal cancer risk.

As a recommendation, biopsy of the colonic mucosa should be routinely taken during the colonoscopic procedure and send for cultivation. Our study followed the standard cultivation practices used in our hospital for the analysis of bacterial species from

SUMMARY BOX

What is known about the subject

- Normal gut bacterial flora play a direct or indirect role in the initiation and progression of tumour by inducing chronic inflammation and producing toxins and metabolites.
- identifying the gut bacterial species involved could be beneficial in understanding their mechanism of action and in preventing the onset of tumour or controlling their advancement.

New findings

- *Clostridium subterminale*, *Finegoldia magna*, *Enterococcus avium*, *Eggerthella lenta*, *Parvimonas micra*, *Clostridium bifermentans*, beta-hemolytic streptococcus, *Streptococcus mutans* and *Morganella morganii* were associated with the risk of developing sigmoid colon cancer while*Proteus mirabilis* and *Morganella morganii* were associated with rectal cancer.
- This study broadens our knowledge on the gut bacterial species involved in colorectal cancer which could be beneficial in understanding their mechanism of action and in preventing the onset of tumour or controlling their advancement.

biopsy samples. The use of molecular techniques, such as 16S rRNA sequencing, would provide a more comprehensive bacterial profiling. Even though no pathology was found during this procedure, identification of certain bacterial which significantly associated with sigmoid colon and rectal cancer can gives us a clue that the patient may have some underlying diseases which predisposed them to the development of malignancy later in life and more frequent colonoscopic screening can be applied to detect the cancer at an early stage. There shall be a study conducted for longer duration with more study samples and to compare the types of bacterial residing both the left and right side of the large bowel. This should include identifying the type of bacterial that present in the tumour involving the right and left colon and to see the difference in total number of bacteria as well as in the diseases that significantly associated with colorectal cancer such as patients with polyps and inflammatory bowel diseases.

From the results of the present study, we can conclude that there were a significant number of bacterial species which can be isolated from mucosa of the large bowel. There were more bacteria present in mucosal of rectum compared to sigmoid colon mucosa with no significant difference in terms of type of bacteria present. There was a slight difference on bacterial type present in normal mucosa of the sigmoid colon and rectum in comparison with the type of bacteria present in sigmoid colon and rectal tumour. *Clostridium subterminale*, *Finegoldia magna*, *Enterococcus avium*, *Eggerthella lenta*, *Parvimonas micra*, *Clostridium bifermentans*, beta haemolytic streptococcus, *Streptococcus mutans* and *Morganella morganii* has been identified as being significantly associated with sigmoid colon cancer while *Proteus mirabilis* and *Morganella morganii* were significantly associated with rectal cancer.

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ORIGINAL ARTICLE

The prevalence of post Covid-19 symptoms & its associated factors in patients at health clinics in Klang Valley, Malaysia

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Background: Individuals often experienced persistence of COVID-19 symptoms following recovery of COVID-19 infection. This study aimed to determine the prevalence of post COVID-19 symptoms and factors associated with its presence (fatigue, depression, anxiety, and stress).

Methods: A quantitative study using a cross-sectional survey was carried out in 14 primary care clinics in 2023. Symptoms of fatigue post COVID-19 were evaluated using the fatigue severity scale (total score of 36 or more indicates fatigue) and presence of depression, anxiety and stress were evaluated using Depression, Anxiety and Stress Scale 21 (DASS-21) (mild severity and above indicates presence of depression, anxiety, and stress).

Result: The prevalence of fatigue, depression, anxiety, and stress in post COVID-19 infection respondents were 54%, 25.8%, 34% and 23% respectively. Respondents who lost the family member(s) due to COVID-19 infection and those who had incomplete vaccination were found to be at risk of fatigue. Depression was higher in younger age respondents, female gender and B40 income group. Anxiety was higher in younger age respondents, people with comorbidities of high cholesterol and heart disease and those with Category 4 and 5 COVID-19 infection; while stress was higher in younger age respondents, female gender and those who lost the family member(s) due to COVID-19 infection.

Conclusion: Fatigue, depression, anxiety, and stress affected almost a quarter of individuals with post COVID-19 infection. By identifying the determinants of these post COVID-19 symptoms, a framework of long-term prognosis of COVID-19 can be drafted to manage these groups of patients more effectively.

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The persistence of COVID-19 symptoms following recovery of the acute infection has been reported widely.[1-2] Fatigue, one of the listed symptoms of post COVID-19, can be due to the physical disease, as well as a manifestation of an underlying psychological disorder, or both. It can be due to health-related and financially distressing conditions of the COVID-19 pandemic, various movement control orders.[3] A study done in Turkey found that 64.1% of participants were categorized as psychologically fatigued and only 35.9% as normal.[4] Using a validated questionnaire, the Fatigue Severity Scale, we are able to gauge the level of fatigue severity in post COVID-19 individual. This has been proven to be a reliable and a valid tool which can be readily used in the local clinical settings.[5] To date, there is possibly limited studies done in Malaysia regarding prevalence of fatigue and its associated factors in post COVID-19 individuals.

On top of this, mental health consequences post COVID-19 infection was found to be significant. One meta-analysis which included nineteen studies identified that stress was the most prevalent mental health consequence (48.1%) of the COVID-19 pandemic, followed by depression (26.9%) and anxiety (21.8%).[6] Quarantine and self-isolation were the main measures adopted to prevent the spread of the disease, resulting in an abrupt change in people's lifestyles, bringing panic and anxiety to a significant number of individuals.[6] These negative psychological effects will eventually lead to significant impact on the individuals and the health care systems thus it is an important aspect that needs to be explored on top of the biological effects of post COVID-19 infection. So far, there is scant data available on the impact of this disease on mental health or the measures taken to limit its spread in the general population or in people who suffer from it including in the local Malaysian healthcare settings. Thus, we would like to not only explore the prevalence of depression, anxiety and stress in post COVID-19 individuals but also importantly identify the associated factors leading to these psychological effects.

The objective of this study is to determine the prevalence of post COVID-19 symptoms among individuals who have recovered from COVID-19 infection and its associated factors with a focus on fatigue, depression, anxiety and stress. This will ensure that screening of at-risk individuals is conducted routinely and early intervention can be taken to prevent further complications especially the interruption of psychological well-being which disturbs the quality of life.[7] Long-term consequences after COVID-19 infection can be an important burden to both society and the health care system.

MATERIALS AND METHODS

This is a cross-sectional study (physical) using self-administered questionnaires in a primary care clinic under district health office in Kepong and Gombak. Respondents are from individuals in the clinic who have a history of COVID-19 infection aged 18 and above. Sample size was calculated by using Kish's formula based on the prevalence of post COVID-19 symptoms of fatigue, depression, anxiety, and stress which was at 70%. The estimated sample size will be 324 with a statistical significance level (α) set at 5 percent. After considering a non-respondent rate of 20%, the estimated final sample size will be 388 participants.

For the study population, the inclusion criteria included adults above 18 years old and Post COVID-19 infected individuals attending primary care clinics under Health District Office Gombak, Selangor and Health District office Kepong, Wilayah Persekutuan Kuala Lumpur. The exclusion criteria were individuals with underlying mental health illness, acutely unwell during the clinic visit, unable to understand English or Malay language and pregnancy. Data collection was done over a six month period from 1st September 2022 until 28 February 2023.

A total of 391 respondents were approached and participated in this study. The response rate was 100% for our study from all the 14 clinics. A convenience sampling was done for post COVID-19 infected individuals attending primary care clinics via face to face.

The pre-tested questionnaire instrument included sections on socio-demography, comorbidities and COVID-19 related factors, post COVID-19 infection symptoms, Fatigue Severity Scale and Depression, Anxiety, Stress Scale-21 (DASS-21). In Fatigue Severity Scale, a total score of 36 and above was considered as having fatigue. A score of mild severity and above in DASS-21 was considered to have depression, anxiety and stress respectively.

Content validation was done with an expert panel of 2 family medicine specialists and 1 infectious disease physician. Pilot study involving 40 participants was done to ensure readability, answerability, and best possible method to conduct the study without interrupting the normal clinic flow.

Participants were selected from the triage counter during regular clinic hours. The nature and objective of study was explained to participants. A patient information sheet was given to the participant. Participants had been ensured that strict confidentiality was maintained. Informed consent was taken before filling the questionnaire. Once a participant consented, he/she was recruited into the study. The participants were told that their participation was anonymous and entirely voluntary. The researcher was around to clarify any doubt(s) that may exist.

Ethics approval was obtained from the Medical Research and Ethics Committee on 9th September 2022 (Approval No. 22-01324-B9U).

Data was analysed using a combination of statistical methods that include simple and multiple logistic regression along with Chi square and Fisher's exact test using SPSS 26.0. Univariate logistic regression test and Chi square/Fisher's exact test was done on socio-demographic, comorbidities, and COVID-19 related factors. For multiple logistic regression, for fatigue the variables that were adjusted were education, presence of family members who passed away due to COVID-19 and vaccination status. For depression, adjusted variables are age, gender, income, category of COVID-19 and heart disease. For Stress, the adjusted variables were age, gender, presence of family members living in the same household infected with COVID-19. For anxiety, the adjusted variables were age, gender, COVID-19 category, hypercholesterolemia, and heart disease. For Fisher's exact, the variables that were analysed are the socio-demographic factors. Variable that had p-value of <0.25 in the univariate analysis were then entered into multivariate analysis to look at the association between socio-demographic factors, comorbidities, and the presence of post COVID-19 symptoms (fatigue and depression, anxiety, and stress) with the p value set at < 0.05.

RESULTS

The mean age participated in this study was 35 years old ±18.0 IQR (Supplemental Table 1 and Supplemental Table 2). Females had the highest number of participants (57.3%). 99.2% of the participants were Malaysians in our study. Malay ethnicity formed the highest number of respondents (78.5%) from the total number of participants followed by Indians (14.3%) and Chinese (5.4%). Almost 57.4% had tertiary education and only 3.1% had primary education. This could be due to our target population which was mainly from the city (Klang Valley). Our respondents were mostly working (75.3%) and had a B40 income status (88.6%). None had any T20 income.

64.7% of the respondents were married. At least 99.7% of the total respondents had one family member who had a history of COVID-19 infection. However, the majority of them survived the illness (94.9%). Most of the participants were non-smokers (84.1%). The mean of the duration of our participants diagnosed with COVID-19 to the data collection is 14 months. 84.9% had COVID-19 category 1 or 2 during the bout of illness while approximately 5% had COVID-19 category 4 or 5 and was admitted for further care in hospital.

Only 19.9% of the total respondents were not vaccinated. Otherwise 35.5% had 2 doses of vaccination and 30.9% had at least the first and second booster dose.

With regards to the post COVID-19 symptoms, 69.9% of the total participants had presence of generalized symptoms from which fever had the highest number of complaints (62.1%) followed by pain the least (40.0%). 52.2% of the respondents complained of presence of ear, nose and throat symptoms from which sore throat was prevalent at 60.3% whereas the least number of complaints was for earache (9.8%).

Presence of respiratory symptoms post COVID-19 infection was high (57.5%) especially cough at 76.8% while only 34.3% from the total reported to have cardiovascular symptoms and gastrointestinal symptoms (37.6%). Almost half of the participants (50.9%) had neurological sequalae following post COVID-19 infection. Out of it, headache formed the

highest complaint at 53.6% while confusion was the least common complaint (8.3%).

Some of the participants complained of psychological symptoms (20.2%) with depression (62.0%) and anxiety (64.5%) being the most common complaint. Approximately 96-97% of the total respondents denied any dermatological symptoms or presence of any other symptoms post COVID-19 infection. 37.1% of the total respondents noted that their quality of life was affected by COVID-19 infection due to the symptoms they had post COVID-19 infection.

In terms of the prevalence of fatigue, depression, anxiety and stress, more than half of the respondents had fatigue (54%), more than a quarter of the respondents had depression (25.8%), 34% of them developed anxiety and 23% of them were reported to have stress.

For the fatigue domain, the odds of developing fatigue are 3 times higher in families which had family members who died due to COVID-19 infection (**Supplemental Table 3** and **Supplemental Table 4**). We also found that those who had incomplete vaccination are at 2.25 times higher odds to develop fatigue compared to those not vaccinated.

For the depression domain, increasing age was associated with a lower odd ratio of developing depression at 0.95 times compared to younger age groups. Females had almost twice the higher odds (AOR=1.85) to develop depression post covid compared to their opposite gender. Those who are in the category of M40 income group had 0.37 times lower odds of having depression compared to the B40 income group.

Age was found to be significantly associated with anxiety. The older age group was associated with lesser anxiety compared to the younger age group (AOR=0.96). Those who were diagnosed with category 4 and higher had almost 3 times higher odds of developing anxiety based on our findings. On the other hand, certain comorbidities such as high cholesterol and heart disease had 3- and 8-times higher odds to develop anxiety respectively (3.44, 8.10= AOR).

Age was also significantly associated with stress. The older age group reported less stress compared to the younger generation (AOR= 0.96). The females are at almost twice higher odds (AOR =1.68) to develop stress post COVID-19 infection compared to the males. Participants who had family member(s) who died due to COVID-19 infection were found to have 3 times higher (3.08=AOR) odds to develop stress in our study.

DISCUSSION

General symptoms

This study identified not only the prevalence of post COVID-19 symptoms of fatigue, depression, anxiety, and stress but also other known post COVID-19 symptoms. COVID-19 patients may have a wide range of clinical manifestations.

Fever as one of the generalized symptoms has been reported to be the major complaint with 58.66% respondents.[9] It is most likely a manifestation of the body's immune response to the viral replication to augment immunity.[9]

As reported in a systematic review, cough as one of the respiratory symptoms had become the most common manifestation with 54.52% respondents. Cough might result from the invasion of vagal sensory neurons by SARS-CoV-2 or a neuro-inflammatory response, or both, leading to peripheral and central hypersensitivity of cough pathways. It improves release of secretions and particles from airways as a result of irritating mechanisms, such as accumulated secretions, postnasal drip, and pathogens, in addition to inflammatory processes.[9-11]

Headache is the most common neurological sequelae of post COVID-19. Possible explanations are direct neuro-invasion with damage to the neuronal pathway and indirect effects mediated by low blood oxygen, high blood pressure and cytokine storm on the central nervous.[10-11]

Fatigue

The presence of fatigue post-COVID-19 affects one's life socially, materially, and spiritually. In this study, more than half of the respondents had fatigue. This prevalence is supported by other studies (Salari et al Mandal et al Mahmud R et aland Ebru Mogul et al where the prevalence of fatigue post-COVID-19 ranging from 45.2% to 69.9%.[3-4,12-13] These shows that fatigue is highly prevalent not only in hospitalized

patients but also in general population post COVID-19 infection. There are multiple causative factors that leads to fatigue post COVID-19 including variety of biological or physical dysfunctions (e.g., genetic factors), cytokines released by SARS-CoV-2 infection that impair psychological defense mechanisms, social factors (e.g., socioeconomic status) as well as psychological factors (e.g., emotional stress).[14]

Our study found that loss of family member(s) due to COVID-19 and incomplete vaccination (received only one dose of COVID-19 vaccine) individuals were at risk of developing fatigue post COVID-19. Loss of family member(s) brings grief which encompasses emotional, cognitive, physical, and behavioral responses which is a common reaction after a loss.[15]

However, people who experienced COVID-19 related grief report more severe grief than people who grieved because of natural losses.[16] Therefore, relatives of patients with COVID-19 felt more burdened by the pandemic compared to those suffering from other illnesses which might explains the higher odds of fatigue in this population.[17]Post-COVID-19 vaccination syndrome could explain the higher prevalence of fatigue in incomplete vaccination compared to unvaccinated individuals. Fatigue was reported to be one of the common, mild to moderate, non-serious side effect of COVID-19 vaccinations.[18]

Participant that was identified to have moderate to severe fatigue (Fatigue Severity Scale score of 4 and above) was referred to the liaison medical officer at the respective study site. During the first follow up, we suggest the medical officer to assess the participant biopsychosocial aspect of fatigue and for non-pharmacological management including low level stretching and strengthening exercise and limiting vigorous intensity physical activity until 2-3 weeks after the post COVID-19 symptoms resolve. We suggest that the participant to be referred to physiotherapist and occupational therapist for education on exercise. We suggest that the participant to be given the next follow up in one month time. During the second follow up, the participant will undergo further evaluation including blood investigations (full blood count, blood sugar (fasting or random) and thyroid function test) if there are persistent or worsening fatigue symptoms and arrange for further follow up. During the next follow up, the participant will be treated if indicated based on blood investigations taken and referred to family medicine specialist of the respective study site or medical physician if persistent or worsening fatigue symptoms despite normal blood investigations for further management and follow up.

Depression

In this study, the prevalence of depression is 25.8%. Depression was reported to be higher in female, younger age respondents and B40 income groups. A study by Charest et al mentioned gender, previous psychiatric history, psychopathology at 1 month follow up and systemic inflammation during acute phase are the main factors associated with depression.[1] Female gender was found to be one of predictors of depression at three-months follow up in a multivariate analysis. Female gender have a higher prevalence of depressive symptoms compared to male gender.[19] Biological differences on the expression of angiotensin-converting enzyme-2 (ACE2) and transmembrane protease serine 2 (TMPRSS2) receptors between males and females, and immunological differences, e.g., lower production of pro-inflammatory interleukin-6 (IL-6) after viral infection in females, explain the higher development of post-COVID symptoms.[20]

Until now there is no study to relate between younger age and depression. Exposure to digital content including mental health illness and being able to report symptoms readily might be the reasons this younger age group had high prevalence of developing depression. Study from a local university in Malaysia found that there is correlation between the B40 income group with depression.[21] This group is more vulnerable to experiencing severe depression. It is a result of economic turmoil that happened during the outbreak that affected around the globe and resulted in retrenchment. Individual concerns about the financial level, unemployment when being fired and the future of the family which disrupt the emotional balance among low-income families.

Anxiety and Stress

A study by Delpino et al which included 2 million participants, shows the general prevalence of anxiety was 35.1%, affecting approximately 851,000 participants. The above data are consistent with 34%

respondents in this study. However, the study also shows there are no significant difference in the prevalence in low and middle-income countries (35.1%; 95% CI: 29.5 % to 41.0%) to high-income countries (34.7%; 95%CI: 29.6 % to 40.1%).[22-24]

One study showed that there are multiple associated factors that lead to severe COVID-19 and mortality including cardiovascular disease. This correlates with this study that showed the association between anxiety post COVID-19 and heart disease.[22-24]

This study also found an association between anxiety and dyslipidemia. It can be explained as they are defaulters during pandemic, thus it increases the risk of getting cardiovascular disease hence increasing the risk of severity, mortality and morbidity of Covid-19 infection. However, further study had to be explored to find association between hyperlipidemia and anxiety in the future.[25]

A local study done among hospitalized COVID-19 patients showed a significant association between the severity of COVID-19 infection with anxiety, whereby category 3 had the highest proportion of anxiety (92.3%) followed by category 5 (90.9%) and category 2 (87.5%), respectively. This correlates with our study which showed a higher proportion of anxiety in category 4 and 5 in comparison with category 1 and 2 COVID-19 infection.[23]

According to the World Health Organization (WHO), anxiety and depression prevalence increased by 25% globally post COVID-19 pandemic.[26] The COVID-19 pandemic has imposed a wide variety of unprecedented challenges, and measures taken to combat the pandemic in the form of lock down, social isolation and many more of which appear to be disproportionately affecting the mental health and well-being of young adults. Anxiety and stress are emotions closely linked with each other. Stress may worsen anxiety and vice versa.

Our study show that younger age group and female gender has higher probability of suffering from stress and anxiety which is similar to one study done at Comenius University in Bratislava.[27] According to another study, those age 16–25 years reported higher stress than those over 45 years old (38.2% vs. 31.8%, $p < 0.05$).[21]

In this study, a higher tendency to anxiety was exhibited by female participants and participants of age group under 30 years of age. We hypothesize that the younger age group are at higher risk for anxiety and stress due to the lower socioeconomic status , poor coping mechanism and social isolation. Generally, late adolescent and adulthood is considered a vulnerable period that coincides with the onset of mental health disorders, such as anxiety and depression.[28] Studies conducted during the COVID-19 pandemic demonstrated strong associations between COVID-19 and COVID-19 related fear and anxiety. Some studies reported higher preponderance of female gender and Covid19 anxiety.[29-30]

Our study showed similar findings of higher psychological effects in female gender. As for female gender, they tend to have more psychological suffering compared to male, most likely since they are involved in the workforce that may be adversely affected by the COVID-19 pandemic, such as retail, service industry, and healthcare.

They also played a significant role in their family by providing emotional and financial support. In addition, the lockdowns had forced most women to stay at home thus resulted in reduced outings, increased home workload, and care for the ill, which contributed to psychological distress.[23,31]

The study also shows respondents with a higher risk perception, lower household income, and poorer health literacy also showed more stress which is reflected in this study as well.[21] In addition, the younger generation who are obligated to work has to stay at home and thus it affects them financially and socially, hence will lead to a stressful environment for them. Studies in Spain, UK, Brazil and Greece reported, elderly people has less psychopathology compared to younger people, less suicidal ideation, lower rates of depression, anxiety and stress. This might be explained by their resilience and complex experiences during previous lives.[32]

This study also found that loss of family member(s) due to COVID-19 leads to stress. This could be explained by the fact that negative emotions of people bereaved by COVID-19 were found worse as compared to non-COVID-19 bereaved. Furthermore,

COVID-19 bereaved relatives had more difficulty recovering from the death of a loved one.[17,33]

Approximately 63% of individuals with sequelae of COVID-19 have difficulty performing daily tasks, self-care, and mobility and have a social and recreational impairment with difficulty returning to work (Ceban et al, 2021).[13,15,34]

Certain socio-demographic factors like ethnicity were not associated with post-COVID symptoms in this study population. In terms of ethnicity, this study was done in Klang Valley where most of the population is multiracial and thus equally exposed to the pandemic. This explains on the non-significant association with the post covid symptoms.

For education, the reason could be probably because the population in Klang Valley is frequently exposed to the latest pandemic news on television, social medias and from the health facility they visit, thus explaining the non-significant finding from this study. Up to date information enables them to stay abreast and reduce uncertainties

In terms of the occupational background, this could be due to the study being done in the Klang Valley and most of them had a stable job and was able to work from home, thus ensuring a stable flow of income. This is contrary to the study done by Mohd Radzniwan et alwhere the study was done in bigger scale in Peninsular Malaysia, Sabah and Sarawak and some had loss of income during the pandemic, contributing to the post covid-19 symptoms.[35] This can be taken as our limitation and future targeted research can be undertaken.

In regards to marital status, we did not find any significant relationship between the marital status and post COVID-19 symptoms as most of our respondents are young adult who are working in Kuala Lumpur. While 2/3rd of our respondents are married and have emotional support from the spouses, the balance is still young adults who are single. 88.6% of the total respondents are also reported to be in the category of B40 (earning <RM 4850). Due to the rise of cost of living, most of the people prefer to stay with family where the support is better. This has been shown in a study done by Mahazril et al where 66% of the adults who are single prefer to stay with family due to the same reason. This could explain the family and emotional support they get, thus the non-significant association with the post Covid-19 symptoms.[36]

Strength and Limitations

There were multiple strengths identified from this study. Firstly, this study was one of the first few studies to determine the prevalence of post COVID-19 symptoms (fatigue, depression, anxiety and stress) and its associated factors. Secondly, this study involved multiple centers in two large districts in Selangor and Kuala Lumpur which constitute a significant local population. Furthermore, participants that were identified to have fatigue, depression, anxiety and stress were referred to the respective medical officer in-charge of the clinic for further intervention including the fatigue management flow chart provided to each study site (14 clinics).

There are a few limitations noted in this study. Even though the study sample was taken from 14 health clinics located within a large urban and suburban population, our study did not cover the rural population thus limiting its generalizability. Furthermore, the cross-sectional design of this study meant that although the associated risk factors of post COVID-19 symptoms (fatigue, depression, anxiety and stress) were identified, its causality cannot be demonstrated. Finally, there was no control group of individuals without prior COVID-19 infection to compare the prevalence of post COVID-19 symptoms (fatigue, depression, anxiety and stress) and its associated factors among the group.

CONCLUSION

This study showed a significant prevalence of fatigue, depression, anxiety, and stress in post COVID-19 infection respondents which were 54%, 25.8%, 34% and 23% respectively. We had identified the risk factors associated with fatigue (loss of family member(s) due to COVID-19 infection and those who had incomplete vaccination), depression (younger age respondents, female gender and B40 income group), anxiety (younger age respondents, people with comorbidities of high cholesterol and heart disease and those with Category 4 and 5 COVID-19 infection) and stress (younger age respondents, female gender and those who lost the family

member(s) due to COVID-19 infection). By identifying these risk factors associated with post COVID-19 symptoms of fatigue, depression, anxiety and stress, framework of long-term effects of these post COVID-19 symptoms can be drawn up respectively to manage these groups of individuals early and effectively.

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ORIGINAL ARTICLE

Lessons Learned from the Dengue Outbreak in Gresik Regency, Indonesia During 2019

Shifa Fauziyah, Budi Utomo, Anik Eko Novitasari, Teguh Hari Sucipto, Nur Fadhilah

A dengue outbreak occurred in Gresik Regency, Indonesia in 2019, the increasing number of cases and also the distribution of cases were used to categorise Gresik Regency as a region that was experiencing an outbreak. This study aims to investigate and identify what lessons can be learned from the outbreak to prevent the next outbreak. An analytical observational study was conducted from January to March 2020. Secondary data, including the number of cases, distribution of cases, larval fee index, and fogging historical activities, were collected from the Health Officer of Gresik Regency. Statistical analysis showed a significant difference in dengue patients' age ($p=0.000$; $p \leq 0.05$). On the other hand, the incidence rate between 2018 and 2019 shows no significant difference ($p=0.406$; $p \leq 0.05$), the distribution of female and male patients ($p=0.239$; $p \leq 0.05$), and the larval indices between 2018 and 2019 ($p=0.296$; $p \leq 0.05$). The fogging activity was conducted based on the occurrence of dengue cases, so the correlation with the number of dengue cases cannot be analysed. Analysis of the incidence rate between 2018 and 2019 shows an increase in the red zone from one district in 2018 to four districts in 2019. Lessons learned from the dengue outbreak in the Gresik regency were the importance of active surveillance, vector controlling in the red zone, regular evaluation of vector resistance status against insecticide, and collaboration between health officers and research institutions.

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Dengue infection remains a significant public health problem in tropics or subtropics countries.[1] Urbanization, poverty, population growth, and mosquito resistance were known as the causes of the rapid spreading of dengue infection.[2,3] Dengue is transmitted by mosquito bite of Aedes species[4]. Dengue virus is classified as genus Flavivirus, family Flaviviridae, and consists of four serotypes: DEN-1, DEN-2, DEN-3, and DEN-4.[5]

The main clinical manifestations of dengue infection include thrombocytopenia, acute febrile illness, and hemoconcentration. Severe cases can lead to hemorrhagic, that can be shown such as petechiae/ skin bleeding, gastrointestinal bleeding, and so on. Symptomatic management of dengue patients, such as fluid replacement therapy can prevent the worst complications.[6]

Dengue infection is rarely confirmed by the laboratory test for the occurrence of viral RNA due to the need for qualified laboratories, especially in developing countries, including Indonesia. The diagnosis was fixed by the physical examination and laboratory test of complete blood cell count.[7] Based on the WHO guidelines shed in 2011, dengue infection can be classified into five groups: DF, DHF I, DHF II, DHF III, and DHF IV. Plasma leakage can be signed with an increasing ratio ≥ 20%, thrombocytopenia less than equal to 100,000/mm^3, and hemorrhagic manifestations. Complications also can appear in hepatic, neurological, and renal.[8]

The first dengue outbreak was reported in Australia in 1897, followed in Greece in 1928, and Taiwan in 1931.[8] Indonesia also experienced a dengue outbreak in some areas, including Bantul.[9] Sleman[10], Palembang[11], Jayapura[12], Merauke[13], and Bandung.[14] The dengue vaccine is still yet to be found, and research is still developing. Some causes of dengue outbreaks have been revealed, such as: vector control, climate change, urbanization, inadequate sanitation, and management of cases.[15] This study aims to investigate what lessons can be learned from the previous outbreak in Gresik Regency in 2019.

METHODS

An analytical observational study was conducted from January to March 2020. This study used secondary data from the health office of the Gresik regency. Data collected consisted of the distribution of dengue infection patients, the number of cases, larval indices, and fogging historical activities. Data were analyzed using SPSS for Windows Version 24.0 (Chicago, IL, USA) and ArcGIS version 10.4. The significance level was p-value ≤ 0.05. The Saphiro-Wilk test was used to test the normality of the data. Due to the abnormality of the data, the Mann-Whitney test was used to compare the data that consisted of two categories, while Kruskall-Wallis was used to compare the data that consisted of more than two categories. Descriptive statistics such as mean and standard error also showed to figuring out the data.

RESULTS

Determination of patients with DF (Dengue Fever) and DHF (Dengue Hemorrhagic Fever) was made based on WHO Guidelines (2009) with the criteria: continuous fever since ≥ 4 days followed by two of the criteria (rash, nausea, vomiting, pains and aches, positive tourniquet test, leukopenia and warning sign). Warning signs consist of persistent vomiting, abdominal pain, lethargy, clinical fluid accumulation, and mucosal bleeding, laboratory test shows an increase in hematocrit and a decrease in platelet count.[8] **Table 1** shows the data recorded through passive surveillance by health officers.

Age and Sex distribution

Table 2 shows the p-value of some variables: age, gender, incidence rate, and larval indices.

The distribution of patients' age was vary, in case, the highest proportion of age was found in 0-10 years old (41.9%), while the lowest was found in 51-60 years old (1.85%) and >60 years old (1.85%). Statistical analysis showed that there is a significant difference in dengue patient's age ($p=0.000$; $p \leq 0.05$), while, the distribution of female and male patients ($p=0.239$; $p \leq 0.05$) shows no significant difference.

Regional differences

Figure 1 shows the difference in incidence rates between 2018 and 2019. There has been an increase in the red zone during the outbreak, as many as three new red zones, namely Gresik district, Kebomas district, and Cerme district. **Figure 2** shows The distribution of dengue cases per month in Gresik regency during an outbreak in 2019.

Table 1 *The distribution of patients, incidence rate, and larval indices in each district in Gresik Regency*

Number	District	Distribution of patients		IR in 2018	IR in 2019	LI in 2018	LI in 2019	Fogging History in 2019
		Female	Male					
1	Gresik	36	37	17.94	65.49	91.83	82.6	35
2	Kebomas	49	36	36.74	86.75	89.04	89	42
3	Manyar	18	35	49.19	57.94	89.36	89	64
4	Bungah	4	3	4.64	4.64	63.5	71	14
5	Balongpanggang	6	4	5.8	5.8	84.86	88.2	29
6	Sidayu	5	4	2.58	3.87	99.49	97	7
7	Duduk Sampeyan	31	7	8.12	19.28	93.59	91.9	7
8	Cerme	45	37	17.87	63.71	73.39	73.1	98
9	Benjeng	25	14	5.21	25.43	84.98	87.5	56
10	Ujung Pangkah	4	2	2.01	3.01	92.45	95.2	0
11	Menganti	4	6	11.94	11.94	80.08	80.4	35
12	Dukun	4	1	1.35	3.38	60.78	66.3	7
13	Driyorejo	7	2	4.08	9.18	96.03	95.1	56
14	Tambak	1	0	0	0.4	97.09	97.3	0
15	Sangkapura	1	0	2.17	0.72	96.88	98.2	0
16	Kedamean	5	2	1.84	4.31	73.07	81.3	14
17	Panceng	2	0	2.58	1.03	94.66	94.7	0
18	Wringin Anom	1	0	2.13	0.71	95.19	95.2	0
Mean	13.77	10.5	9.78	20.4	86.4	87.3	25.78	

Table 2 *Statistical analyses of age, gender, incidence rate, and larval indices while the dengue outbreak in Gresik Regency during 2019*

Variables	Mean ± SE	*p*-value
Age*		
0-10 years (41.9%)	10.06±2.83	
11-20 years (34.5%)	8.28±2.33	
21-30 years (11.8%)	2.83±1.03	
31-40 years (3.94%)	0.94±0.35	***< 0.0001***
41-50 years (3.94%)	0.94±0.48	
51-60 years (1.85%)	0.44±0.25	
>60 years (1.85%)	0.44±0.24	
Gender**		
Female	13.78±3.8	0.23
Male	10.56±3.42	
Incidence Rate**		
2018	9.78±3.14	0.4
2019	20.42±6.53	
Larval Indices**		
2018	86.45±2.76	0.29
2019	87.38±2.28	
Fogging Activity	25.78±6.68	-

Incidence Rate

This study highlighted the increasing incidence rate of dengue infection in Gresik regency, as many as 10.64 per 100,000 inhabitants compared with previous years (Table 1). According to the guidelines from the Indonesian Ministry of Health, the value of incidence rate can figure out zone classification. In 2018, only the Manyar district that classified as a red zone due to the number of IR above 49, whereas in 2019, there was an increasing number of red zones, becoming four districts. Based on Table 1, the highest incidence rate was found in the Kebomas district (86.75), while the lowest was found in the Tambak district (0.4). The incidence rate between 2018 and 2019 shows no significant difference ($p=0.406$; $p \leq 0.05$).

Larval indices

Larval indices consist of the house index (HI), Bretau index (BI), and container index (CI). Larval indices show that only five districts reached the target of larval indices ≥95%, namely Sangkapura district,

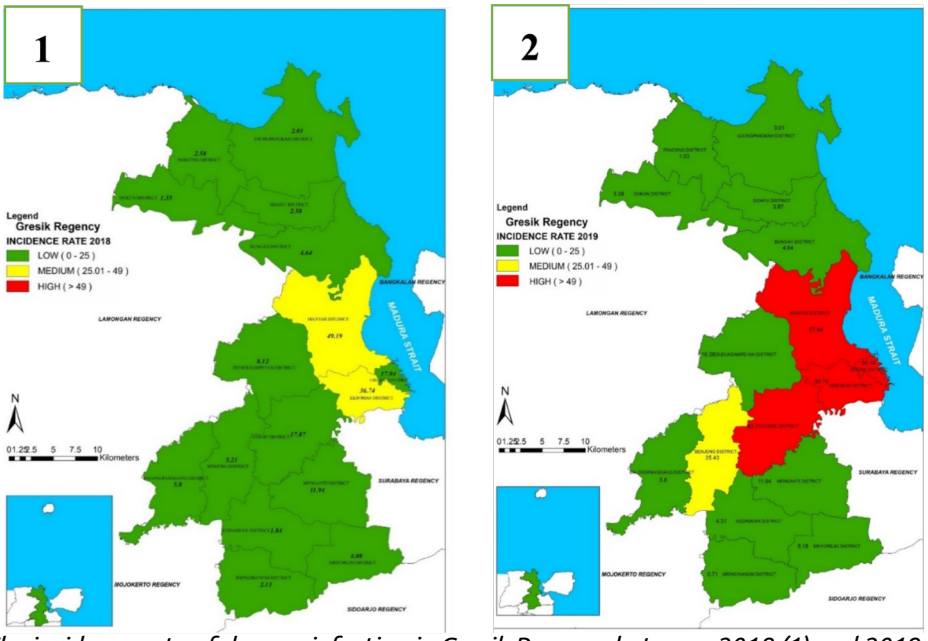


Figure 1 *The incidence rate of dengue infection in Gresik Regency between 2018 (1) and 2019 (2)*

which had the highest larval indices (98.2%), while, the lowest larval indices reported by Bungah district (71%). Overall, the number of larval indices in the Gresik regency is still below the national target and needs to be gained. There is no different significant in the larval indices between 2018 and 2019 (p=0.296; p≤0.05) (Table 2).

Fogging activity

The fogging activity was conducted based on the occurrence of dengue cases, so that cannot be analysed the correlation with the number of dengue cases (Table 2). The number of dengue cases every month is shown in Figure 2 below. The most fogging activities were found in the Cerme district (98 times fogging). In contrast, the lowest was found in Ujung Pangkah, Tambak, Sangkapura, Panceng, and Wringin Anom districts (0 fogging time).

DISCUSSION

This study highlighted some points related to the dengue outbreak in 2019 in Gresik Regency. The majority of cases occurred in children from 0 to 10 years old. There is an increasing incidence rate between 2018 and 2019, as many as 10.64, but there's no significant difference between the incidence rate in 2018 and 2019. The larval indices were increased to the previous year, as many as 0.93. At the same time, a significant difference was not detected—fogging activity based on the number of cases and the regulation from the health officer.

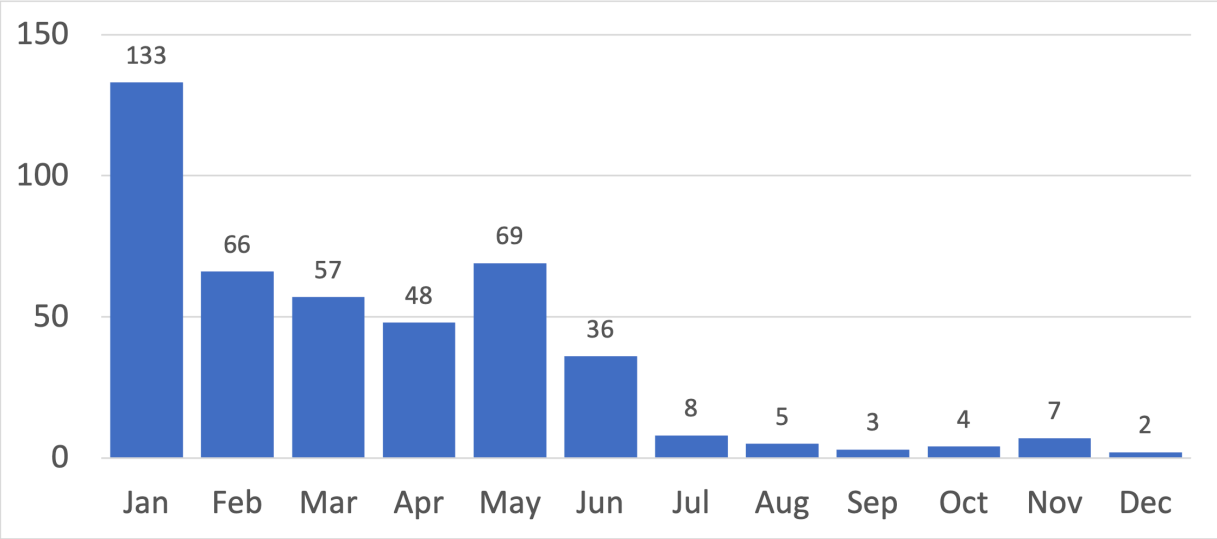


Figure 2 *The distribution of dengue cases per month in Gresik regency during an outbreak in 2019*

Female patients were 1.3 times higher than male patients (1.3:1), with no significant difference between the female and male gender.

This study's epidemiological characteristics show that children ≤ 10 years old were the most infected group. This phenomenon was in line with a report in another country in the South East Asia region that shows children tend to be infected by dengue infection more than adolescents and adults.[16,17] On the other hand, severe clinical manifestation was found in adults, especially in the tropics area.[18] Age has been known as a risk factor for dengue infection, some settings show that children tend to be more infected than the elderly[19,20], but in another setting, the elderly group was reported as the highest risk group.[21] Promoting healthy behavior in school would be the best practice to prevent children-age school infected by dengue. DENV-2 and DENV-3 serotypes had been detected in some elementary schools in Yogyakarta, supported by other environmental factors such as temperature, humidity, larval indices ventilation, and distance between other buildings.[22] The dengue serotype that circulates in Gresik regency is yet to be identified, especially in school areas with the possibility as high-risk areas. Detection of spread serotypes in high-risk areas can be an early warning system to prevent the potential of the next outbreak. In this case, the collaboration between the health worker, policy maker, and the school's owner should be made.

From this study, the peak incidence was reported in January, which means in the rainy season. Rainy seasons have been known as a risk factor for increasing dengue cases.[23,24] Vector control through observation of the larval abundance was also an important program conducted by environmental health units in every district. This program is monitoring the abundance of dengue vectors. Larval indices consisting of house index (HI), Breteau index (BI), and container index (CI) was calculated to reduce the number of breeding site of dengue vector. These indices have been used widely.[25] The average of all three indices is counted and stated as larval indices. Larval indices in 2019 increased as much as 0.93 compared with the previous year, but this number is still yet to reach the national target of 95%. Of these, they indicated a district's vulnerability to dengue transmission. The Bruteau index has been recommended as a controlling program and significantly influences predicting the transmission of dengue infection, with a sensitivity of 78% and a specificity of 63%.[26] Otherwise, monitoring fundamental premises and containers more revealed container productivity so that the transmission can be reduced.[27]

The other vector control program the health officer conducted during the outbreak was fogging activity. Our interview with Health Officer revealed that fogging training was conducted based on the reported cases. When the dengue cases are reported, the closest public health care worker will bear thermal fogging spray or ultra-low-volume (ULV) spray using malathion 0.05%. Malathion is classified as organophosphate insecticide.[28] This vector control aimed to kill adult mosquitoes to minimize the second transmission.[29] However, long-term usage of this method remained another problem, such as mosquito resistance. The mosquito resistance test is yet to be conducted in the Gresik regency, so the vector status against insecticide hasn't been provided. Organophosphate insecticides have been used as thermal fogging spray for decades in Indonesia[30], and some mosquito resistance has been reported.[31–34] Evaluating mosquito resistance status is essential due to the precise goals. Annual controlling of mosquito resistance status in Gresik regency can be conducted with research institutes that have experience since the mosquito resistance test must be completed by trained personnel.

The number of female genders in this study was higher than male patients. This result was in contrast with another study reported in Asia that shows male dengue patients were more predominant than females because males likely spend more time outside than females.[35,36] The difference in gender of dengue patients can be caused by some factors such as genetic, hormonal factors, immunological, and activity that can affect the susceptibility to disease of individual.[37] Other than that, the severity of dengue patients was also supported by another factor, such as the interaction between population dynamics and viral genetics.[38]

Analysis of the changing incidence rate in endemic areas of dengue infection should be conducted due to mapping high-risk and low-risk areas. The increasing incidence rate in some geographical

regions can be influenced by factors such as urbanization, modern transportation, mobility of citizens, and population growth.[39,40] The geographical mapping in this study was made based on the reported cases to the health officer, or in other words, called passive surveillance. The dengue surveillance model in Indonesia should adopt another country in South East Asia that already has active surveillance, such as Singapore and Malaysia.[41] In some cases, data from passive surveillance can lead to underreported/underestimated.[42] Active surveillance can detect, estimate, and predict the increase in dengue cases, so policymakers can use the information to allocate logistics. Precisely expecting the increasing instances can prevent the outbreaks.[43]

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CONCLUSION

Statistical analysis showed a significant difference in dengue patients' age ($p=0.000$; $p \leq 0.05$). On the other hand, the incidence rate between 2018 and 2019 shows no significant difference ($p=0.406$; $p \leq 0.05$), the distribution of female and male patients ($p=0.239$; $p \leq 0.05$), and the larval indices between 2018 and 2019 ($p=0.296$; $p \leq 0.05$). Lessons learned from the dengue outbreak in the Gresik regency were the importance of active surveillance, vector controlling in the red zone, regular evaluation of vector resistance status against insecticide, and collaboration between health officers and research institutions.

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CASE REPORT

A rare case of an intra-scrotal Amyand's hernia

Thomas Saliba, Giulia Negro

Background: Amyand's hernias are rare forms of inguinal hernias in which the appendix is contained within the hernia, being present in a minority of hernias. Clinical diagnosis is difficult, with most cases being found intra-operatively as ultrasound is rarely performed beforehand.

Case report: A 1-month-old premature male infant was referred to our emergency services with a right-sided inguinal mass suspected to be an inguino-scrotal hernia. The mass was clinically non-tender and non-inflamed. An ultrasound exam of the right inguinal mass was performed, revealing a non-inflamed appendix alongside a normal appearing testicle, with a small amount of hydrocele. Surgical intervention was subsequently undertaken to address the hernia.

Discussion: Amyand's hernia presents a unique challenge in diagnosis and management. Our patient's case corresponded to Amyand's hernia type 1 and stage 3, as per the Losanoff and Basson classification, where the appendix is non-inflamed and has descended to the scrotum. This pathology is exceedingly rare, with the appendix being found in approximately 1% of inguinal hernias, predominantly in males. The pathogenesis is not fully understood but is attributed to fibrous connections and a patent process vaginalis. The diagnosis is often made intra-operatively due to the variable clinical presentation.

Conclusion: We present a rare case 1-month-old premature boy, who presented with a right inguinal mass. Ultrasound revealed an intrascrotal type 1, stage 3 Amyands's hernia.

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Amyand's hernias are rare forms of inguinal hernias in which the appendix is contained within the hernia.[1] The incidence of this pathology is around 1% of all inguinal hernias.[1,2] The appendix contained within the hernia can either be non-inflamed, inflamed or even perforated, which can be further classified according to Losanoff and Basson's classification.[3] The pathophysiology is still debated and, as it is difficult to diagnose it pre-operatively, most cases are discovered during operations for inguinal hernias.[4] We present an exceedingly rare case of an Amyand's hernia in a 1-month-old boy in which the appendix descended fully into the scrotum.

CASE REPORT

A 1-month-old boy, prematurely born at 36 weeks gestation, was referred to the emergency services of the hospital by his pediatrician for a right sided inguinal mass, with suspected inguino-scrotal hernial. Upon examination by the on-call surgeon, the patient was referred to radiology in order to rule out a contralateral hernia before surgery. The right inguinal mass was not tender, inflamed, or hard to the touch. Ultrasound of the left inguinal area was unremarkable, with a small hydrocele around a normal left testicle. Ultrasound of the right inguinal mass revealed it to be an inguino-scrotal hernial containing bowel loops. The right scrotal sack revealed a hydrocele, a normal appearing testicle and bowels (**Figure 1** and **Figure 2**). Furthermore, the appendix was also present within the scrotum, looping inferiorly to the testicle (**Supplemental Video 1**). The appendix was of normal thickness and contained fecal matter but no appendicolith. The patient went on to be operated upon in order to resolve the hernia.

DISCUSSION

Amyand's hernia is a rare diagnosis where the appendix is located in an inguinal hernial sac, named after Claudius Amyand who first described the pathology in 1735.[1,5] The incarcerated appendix may either be normal or inflamed.[5] Our patient presented with an Amyand's hernia type 1 and stage 3, according to Losanoff and Basson's classification, defined as an inguinal hernia involving the caecal appendix without signs of inflammation which has reached the scrotum.[1,6] Approximately 1% of inguinal hernias contain the caecal appendix, making such presentations rare, with a higher incidence of right inguinal hernias, with which it is associated.[1] There is a male preponderance to the pathology with a 90% of overall cases being found in males and a 28:1 ratio of males to females in pediatric populations.[4] However, it has also been noted that in the rare cases of neonatal appendicitis, which represent around 0,1% of infantile cases, the appendix is within the inguinal canal in up to a third of cases.[5] With regards to the pathogenesis, it has been suggested that a fibrous connection between the appendix and the testicles,

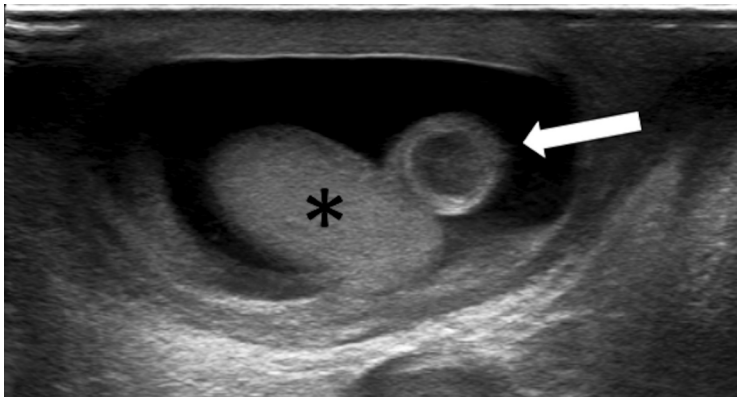


Figure 1 *Ultrasound image acquired using a linear probe showing the scrotum of the patient containing the right testicle (asterisk) as well as the appendix (arrow), seen in a cross-sectional view. The patient also has a small hydrocele surrounding both the testicle and the appendix.*

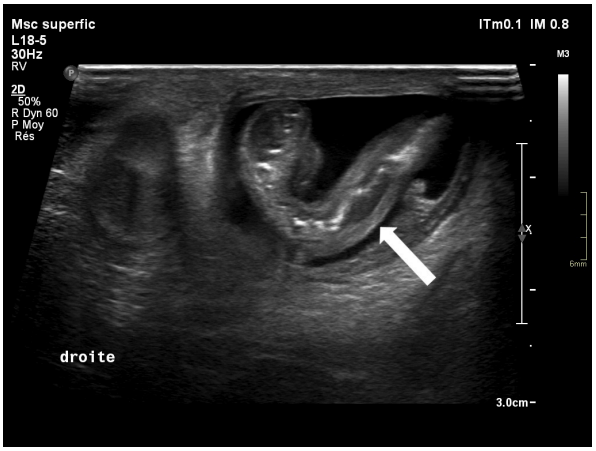


Figure 2 *Ultrasound image acquired using a linear probe showing the scrotum of the patient containing showing a longitudinal view of the appendix (arrow), which is clearly visible, containing a small amount of air and fecal material. The patient's hydrocele is also visible.*

in addition to a patent process vaginalis, could lead to the appendix being pulled into the inguinal canal, which would account for the higher incidence in children and premature infants.[1] Most Amyand's hernias are right sided, however left sided Amyand's hernias have been reported.[1] This pathology is difficult to diagnose due to having variable symptoms depending on the state of the appendix, though our case shows it can also be an incidental finding, with physical examinations and laboratory results sometimes being non-contributive to the diagnosis.[1,5] In patients who are symptomatic, it has been described as mimicking an incarcerated or strangulated hernia.[5] As imagery is generally not requested by surgeons unless the inguinal hernia is incarcerated or resists reduction, Amyand's hernia remains an uncommon ultrasound finding, with the diagnosis generally being made intra-operatively.[1]

With regard to the management of the condition, recent studies have pointed towards laparoscopy being the preferred approach as it allows for better visualization of the entire abdominal cavity and is thus both diagnostic and therapeutic.[5] Concurrent appendectomy and inguinal hernia repair is possible, but the indication to do so is remains debated.[1,5]

Although other reports of type 1 Amyand hernias exist in the literature, we found no others describing an intrascrotal appendix in a neonatal case, leading us to believe that this a very rare occurrence.

CONCLUSION

We presented the case of a 1-month-old boy who presented for the investigation of a suspected inguino-scrotal hernia in whom an Amyand's hernia was discovered. Amyand's hernias are rare forms of inguinal hernias containing the appendix. They are often discovered during surgery because they are difficult to detect clinically, often being mistaken for incarcerated hernias. Their pathophysiology remains poorly understood and the indications for the type of surgical intervention are also debated.

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CASE REPORT

TEVAR for blunt thoracic aortic injury - Case Report

Glenn Briffa, Kevin Cassar, Joseph Galea, Matthew Joe Grima

Blunt thoracic aortic injuries (BTAIs) stand as a prominent cause of mortality in trauma scenarios due to high-energy blunt trauma, such as in motor vehicle accidents. Thoracic Endovascular Aortic Repair (TEVAR) has emerged as the preferred treatment modality for BTAIs owing to its minimally invasive approach and superior outcomes compared to open surgery. Here, we present the case of a 31-year-old woman involved in a severe motor vehicle accident, resulting in acute thoracic aortic injury managed with TEVAR in Mater Dei hospital.

Upon admission, the patient demonstrated signs of hemodynamic instability, prompting urgent CT imaging which revealed aortic injury alongside other traumatic injuries. Emergency TEVAR was successfully executed under general anesthesia, followed by revascularization for a concurrent popliteal artery injury. Subsequent imaging confirmed proper stent positioning and resolution of the aortic injury. The patient experienced a satisfactory recovery with a hospital stay lasting 25 days.

Diagnosing BTAIs poses challenges due to their nonspecific clinical manifestations, necessitating reliance on imaging modalities, primarily computed tomographic angiography (CTA). TEVAR offers distinct advantages over open surgery, including reduced invasiveness, shorter operative times, and decreased postoperative complications. However, long-term surveillance remains crucial due to potential complications. TEVAR persists as the primary treatment modality for eligible BTAIs, ensuring favorable patient outcomes and enhanced recovery.

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Autopsy based studies indicate that blunt thoracic aortic injury (BTAI) is the second most common cause of death in trauma cases in young adults, preceded only by head injury.[1] The two most frequently noted factors contributing to BTAIs are high energy blunt trauma and rapid deceleration. Motor vehicle accidents, especially head on collisions, account for 81% of cases of BTAIs.[2] BTAIs are defined as a tear in any part of the aorta that arises secondary to a combination of stretch and shear forces, precipitate deceleration, raised intravascular pressures and compression of the aorta between the anterior chest wall and the vertebral column.[3] These injuries may occur within any part of the aorta, from the aortic root to the common iliac artery bifurcation, however, are more common at sites of aortic tethering, such as the aortic isthmus, as shear forces tend to act more vigorously at such points.[4]

The mainstay treatment of BTAIs, barring any contraindications, is now considered to be Thoracic Endovascular Aortic Repair (TEVAR). TEVAR confers superior outcomes to open surgery due to its minimally invasive approach and associated lower complication rates as well as a shorter recovery period.

We present the case of a 31 year-old lady who presented to the emergency department after a high impact motor vehicle accident, who sustained acute thoracic aortic injury and was treated with TEVAR.

CASE REPORT

A 31 year-old woman who was previously healthy, was brought to the emergency department by ambulance after being involved in a high impact motor vehicle accident between her car and a heavy truck. The patient was found unresponsive in the drivers' seat with the steering wheel impacted into her chest and both lower limbs entrapped. She endured a difficult extraction and was given intraosseous fluids, her spine was immobilized and a pelvic splint was applied. Tranexemic acid was given intravenously and oxygen was administered via a non-rebreather mask on site. She regained consciousness after on-site resuscitation with fluids and oxygen.

Upon initial primary survey, her airway was patent with no obvious signs of obstruction. She was tachypneic with oxygen saturations of 99% on non-rebreather mask, her radial pulse was thready on the right side but her carotid pulse was palpable on the same side. Her blood pressure was 126/75 but tachycardic. She was otherwise hemodynamically stable. Focused assessment with sonography for trauma (FAST scan) was performed and free fluid was noted in the right upper quadrant and left upper quadrant of her abdomen. She was noted to have a Glasgow coma scale of fourteen but was at times agitated and confused.

She was transferred to the radiology department for a CT-Polytrauma which revealed an abnormal contour of the proximal descending aorta just distal to the origin of the left subclavian artery which was associated with luminal filling defects and massive surrounding loss of planes in the mediastinum, suggestive of a large mediastinal haematoma which spread caudally along the abdominal aorta. Evidence of pulmonary embolism was noted in the lower branch of the right pulmonary artery with a small right sided pneumothorax. The abdominal aorta was also noted to have a decreased diameter with an adjacent collapsed inferior vena cava and hypoperfusion defects in both kidneys. A comminuted fracture of the middle third of the right femur was also confirmed on CT.

The major trauma protocol was initiated and the patient was transferred directly to the operating theatre for a TEVAR.

Emergency planning was performed to size the defect and select the appropriate stent size. The procedure was performed under general anesthesia and broad spectrum prophylactic antibiotics were administered intravenously. Ultrasound guided percutaneous access to the left common femoral artery was achieved and a stiff Lunderquist wire was placed in the ascending aorta. A pigtail vascular catheter was inserted percutaneously through the right common femoral artery and placed in the aortic arch. On-table angiography was performed and the left subclavian artery orifice was marked and the aortic lesion identified. A proximal freeflow straight Valiant stent graft 22-22-112 (Medtronic) was inserted in the arch of the aorta and an on-table angiography was performed to confirm correct position relative to the left subclavian artery origin and the stent deployed beyond the origin. A post-deployment angiogram

Table 1 *Stanford Classification*

Stanford Type	Definition
Type A	Involves the ascending aorta alone, or in addition to the arch and descending aorta
Type B	Involves the descending aorta alone while sparing the ascending aorta and arch
Type Non-A Non-B	Involves the arch and descending aorta but not the ascending aorta

confirmed correct positioning and flow through the stent-graft. Proglide closure system (Abbott Cardiovascular) was used to close the common femoral artery access site.

Immediately postoperatively, the patient's right foot was cold and pale despite normal movement. Review of the CT-Aorta revealed a right popliteal artery injury requiring revascularization. The patient underwent a successful right femoral to popliteal artery bypass procedure using ipsilateral great saphenous vein.

Follow up CT-Aorta performed on the following day confirmed that the stent was positioned correctly, the walls of the thoracic aorta were smooth with no residual internal dissection flaps. The branches of the aortic arch were widely patent and did not contain any dissection flaps. A repeat CT-Aorta performed one month post-TEVAR confirmed correct stent-graft position with patent left subclavian artery and complete sealing of the wall defect.

The patient remained in hospital for a total duration of twenty five days for the management of other injuries and complications of the trauma sustained. Follow-up scan at one year was unremarkable and the patient was doing well and eager to start a family.

DISCUSSION

Clinical Presentation of BTAIs

Blunt thoracic aortic injuries are not reliably diagnosed clinically due to the variability and non-specificity of the clinical signs and symptoms. BTAIs are commonly recognisable clinically at the point where hemodynamic instability develops secondary to aortic failure. The most commonly observed clinical signs include: upper limb hypertension and lower limb hypotension, systemic hypotension and discrepancy in blood pressure between the left and right brachial arteries. Advanced Trauma Life Support (ATLS) algorithm should be followed in managing such trauma patients due to the high likelihood of multiple associated injuries.[5]

Imaging

Chest x-rays (CXR) are widely used as an adjunct to the primary survey in the ATLS algorithm and multiple findings through this modality may suggest the presence of BTAIs. Such findings include but are not limited to: widening of the mediastinum, obliteration of the aortic knuckle, apical capping due to blood in the apex, sizeable left sided pleural effusions representing an underlying haemothorax and displacement of the left mainstem bronchus. Focused assessment with sonography in trauma (FAST) scanning may also give valuable information to support the presence of BTAIs such as the presence of a left haemothorax and pericardial tamponade in the case of free rupture of the aorta. Unfortunately, both CXR and FAST findings have very poor specificity and sensitivity and they may only heighten the index of suspicion of BTAIs without confirming or excluding their presence and without contributing to the planning of surgical management.

The gold-standard imaging modality to diagnose BTAIs remains computed tomographic angiography (CTA).[6] The most common finding on CTA which suggests an aortic dissection is the presence of an intimal flap which is depicted as a linear filling defect within the aorta, typically due to an intimal tear. Damage to the tunica intima as well as the tunica media may be depicted by pseudoaneurysm formation. If damage to the tunica adventitia occurs, implying that a transmural tear of the aorta is present, contrast may appear to be extravasating.

Classification and Grading of BTAIs

Traditionally, aortic dissections are classified using the Stanford system (Table 1) which separates these pathologies into Type A, Type B and Non-type A Non-

Table 2 *Grading of BTAIs*

Grade of Injury	Determinants
1	Intimal Tear
2	Intramural Haematoma
3	Pseudoaneurysm
4	Rupture

type B depending on the extension of dissection in relation to the left subclavian artery.[12]

The most widely used grading system for BTAIs is depicted below (Table 2) and endorsed by both the Society for Vascular Surgery (SVS) and the European Society for Vascular Surgery (ESVS).

Management

The ESVS clinical practice guidelines for the treatment of BTAIs suggests that operative intervention, either in the form of open repair, or ideally TEVAR when not contraindicated, should be considered for most cases of BTAI. It is suggested that only minimal aortic injuries presenting as an intimal tear, be eligible for expectant management with serial imaging. These cases account for only ten percent of BTAIs.

If operative intervention is indicated, the data suggests no significant difference in mortality between immediate intervention and delayed intervention, hence most recent guidelines allow for delayed intervention in the case of other life threatening injuries requiring intervention or medical attention.[13]

Conservative Management

Once BTAI is suspected or confirmed, tight blood pressure control and heart rate control should be achieved to prevent rupture or end-organ damage. Systolic blood pressure should be kept below 100 mmHg and heart rate less than 100 bpm. Beta blockade is the strategy of choice in controlling blood pressure and heart rate unless contraindicated. Calcium channel blockers or arterial vasodilators are used when additional supplementation is necessary. Antiplatelet therapy and anticoagulation also form part of initial management of BTAIs.

A CTA should be repeated within the first 48 to 72 hours when treating BTAIs conservatively to confirm stability and exclude extension.[8]

Open versus endovascular repair

Open repair typically involves a high posterolateral thoracotomy in the majority of injury patterns. This is a highly invasive procedure which may exacerbate other injuries incurred during the trauma and lead to significant morbidity and mortality. In open approaches, an interposition graft is sewn over the dissected segment while a bypass circuit is used to perfuse distal organs. Commonly encountered complications are: major blood loss, paraplegia, stroke and other ischemic injuries.[9]

TEVAR confers a much less invasive approach which has revolutionized the treatment of BTAIs. It is worth noting that not all BTAIs are eligible for TEVAR. Candidacy depends on the anatomy of the patient and the exact nature of the injury. Open surgery may still be necessary due to anatomical features that prohibit an endovascular approach. Open repair is nowadays viewed as a complementary treatment modality to endovascular intervention as opposed to a competing approach.[10]

CONCLUSION

TEVAR has many advantages over open surgical approach, notably the avoidance of invasive measures, such as open thoracotomy, single lung ventilation, aortic cross-clamping and the associated significant haemodynamic changes as well as the requirement for cardiopulmonary bypass. The endovascular approach also confers a shorter operative time and postoperative hospital stay while inflicting less trauma on a multi-trauma patient.

The main disadvantage of TEVAR is the need for long-term and potentially life-long radiological surveillance due to the risk of endograft collapse in case of oversizing as well as endograft migration and endoleak secondary to the natural growth of the aorta in younger individuals. This exposes patients to significant radiation and may be a limiting factor for patients living in rural areas where radiological imaging is not readily available.

In acute type B aortic dissections, follow-up scans are recommended at three and six months and then yearly for three years. If in stable condition, the interval may be increased to two to three years. If aortic growth is more than five millimeters per year or aortic diameter is more than fifty millimeters, increased monitoring or intervention are warranted. However, the evidence supporting these guidelines is limited.[13]

TEVAR is now the main treatment modality for eligible BTAIs in Mater Dei hospital as in the case reported here. Review of local data is recommended to ensure that outcomes are comparable to those in other international centers.

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CASE REPORT

A Case of Severe Pulmonary Stenosis for Planned Caesarean Section with Epidural Anaesthesia

Christian Camilleri, Kevin Crockford

Background: Pulmonary stenosis (PS) is a congenital heart defect that can present in a variety of clinical settings, depending on the severity. To ensure both maternal and fetal well-being, the management of PS in pregnant patients requires careful multidisciplinary coordination and consideration of both normal changes in maternal physiology and abnormal changes related to comorbidities.

Case Presentation: We present a 40-year-old female with a history of severe congenital pulmonary stenosis, hypertension, and diabetes mellitus, who underwent an elective caesarean section with tubal ligation under regional anaesthesia. She was diagnosed with PS in early childhood but was later lost to cardiology follow up. During antenatal assessment, trans-thoracic echocardiography revealed severe pulmonary stenosis with a gradient of 100mmHg, with severe right ventricular dilation. The patient maintained good exercise tolerance and had no symptoms suggestive of heart failure.

Intervention and Outcome: The caesarean section was conducted under lumbar epidural anaesthesia with careful monitoring of her cardiovascular status, including radial artery invasive blood pressure monitoring and CVP through a central venous catheter. Mild sedation was provided with boluses of midazolam. Postoperative recovery was uneventful, with stable blood pressure and mild improvement in the pulmonary gradient.

Conclusion: Epidural anaesthesia is a safe option for patients with severe PS undergoing Caesarean section provided that the block is achieved slowly to avoid rapid changes in blood pressure.

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Pulmonary stenosis (PS) is a congenital heart defect that involves obstruction to the right ventricular outflow tract. Severe PS, defined as a peak gradient >64mmHg, is often associated with right ventricular hypertrophy, dilation of the right atrium, and in extreme cases, heart failure. Pregnant women with PS are at increased risk due to the additional strain placed on the cardiovascular system during pregnancy and labour. A careful and coordinated approach involving multiple specialties, including cardiology, obstetrics, and anaesthesia, is essential in these cases to mitigate the risks to both mother and fetus.

CASE PRESENTATION

A 40-year-old female, referred to as Patient X, with a known history of congenital severe pulmonary stenosis (PS), hypertension, non-insulin dependent diabetes mellitus, and anxiety disorder, presented at 36 weeks of gestation for an elective caesarean section with tubal ligation. The patient has a significant smoking history. The patient's maternal family history includes cerebrovascular accidents (CVA). This was the second pregnancy for Patient X. Her first pregnancy, 12 years prior, resulted in a normal vaginal delivery with no complications.

The pulmonary stenosis was diagnosed in childhood, with early echocardiograms demonstrating mild PS, but no significant stenosis or post-stenotic dilatation. She was subsequently lost to cardiology follow-up. A few months prior, a trans-thoracic echocardiogram revealed severe pulmonary stenosis with a gradient of 100 mmHg, severe right ventricular dilation, and right atrial enlargement. She had a loud ejection systolic murmur.

Her regular medications included Aspirin, Sertraline and Enoxaparin.

The patient was classified as ASA 3. Her BMI was 42, she had a short neck, and a Mallampati score of 2. A loud ejection systolic murmur (ESM) was audible.

Epidural anaesthesia was selected as the mode of anaesthesia with careful build-up of the block to avoid the airway risks of general anaesthesia and minimise cardiac instability. The epidural was placed at the L2/3 interspace under sterile technique, with loss of resistance to saline. A test dose of 0.5% bupivacaine was administered to confirm correct placement, followed by a total dose of 20 ml of 0.5% bupivacaine and 2 ml of 50 mcg/ml fentanyl by slow incremental boluses given over a period of 30 minutes.

Blood pressure was measured via a radial arterial line. A right jugular central venous catheter was placed with ultrasound guidance in case vasopressor infusions were needed.

Medications Administered in Theatre

- Midazolam IV 2mg upon arrival followed by 1mg 30 minutes later
- Tranexamic Acid 1g IV
- Co-Amoxiclav 1.2g IV
- Dexamethasone 8mg IV
- Syntocinon 2 units over 10 minutes after umbilical cord clamping, followed by an infusion of 2.88 units in 500 mL Ringer's lactate at 125 mL/hr.
- Misoprostol 1g PR

Surgical Procedure

The patient underwent a lower segment caesarean section (LSCS) with concurrent tubal ligation. Skin incision was performed after confirmation of adequate block to T4 and fetal delivery was at 17 minutes post-skin incision. A healthy female infant was delivered, who cried on initial stimulation, with an Apgar score of 9 at 1 minute and 9 at 5 minutes. The estimated blood loss by completion of skin closure was estimated at less than 200ml.

Postoperative Management

Patient X remained haemodynamically stable, with no signs of acute decompensated heart failure or bleeding or other complications. She was admitted to central delivery suite for monitoring for 24 hours after which the arterial line, central venous catheter and epidural catheter were removed. Postoperative echocardiography showed stable cardiac function despite the surgical stress.

She was discharged home after 8 days and advised to follow up with her obstetrician, cardiologist, diabetologist and psychiatrist. She was also followed up by outpatient community clinic nurse-led wound care for delayed wound healing.

DISCUSSION

In contrast to mitral and aortic stenosis, PS without other major cardiac abnormalities or symptoms of decompensation does not usually adversely impact maternal or fetal outcomes of pregnancy.[1]

Factors that could contribute to cardiac decompensation in this setting include:

- The Epidural sympathetic block resulting in drop in SVR, preload and mean arterial pressure. In stenotic lesions, the cardiac output may not increase to compensate for reductions in SVR, resulting in a precipitous decline in MAP.(2)
- Patient Anxiety resulting in sympathetic-mediated tachycardia, increased myocardial oxygen consumption and coronary vasospasm
- Blood loss resulting in hypovolaemia and a drop in mean arterial pressure
- Uterotonic drugs affecting systemic and pulmonary vascular tone.

Other case reports suggest neuraxial anaesthesia for caesarean section in patients with PS is safe.[3-5]

Intraoperative management of patients with stenotic lesions such as pulmonary stenosis usually aims for a slower heart rate in the lower range of normal to allow adequate timing for ventricular filling and ejection.(5) This is one benefit of the use of phenylephrine as a vasopressor over ephedrine in this case, as the former tends to lower the heart rate while the latter increases it.

Uterotonic Drugs

The adjustment of uterotonic agents to minimize impact on the pulmonary vasculature had to be weighed against the need to minimise post-operative blood loss which would adversely affect the mean arterial pressure.

Oxytocin/syntocinon results in systemic vasodilation, hypotension, tachycardia and increased cardiac output.[6] Therefore, the minimum effective dose should be used.

Ergometrine[7] and Carboprost (prostaglandin PGF2-a)[8] both cause increased pulmonary artery pressures and were therefore avoided. Carboprost is not recommended in the context of significant cardiac disease.[9]

Misoprostol is less effective than oxytocin in preventing postpartum haemorrhage, but its safety profile makes it a useful adjunct.[9]

Obstetric Planning

Planned caesarean section was decided over vaginal delivery to avoid Valsalva during labour which decreases preload and could lead to cardiac decompensation. Balloon valvuloplasty was not indicated as exercise tolerance was good. If decompensation occured, the hospital is equipped to perform open heart surgery to correct the lesion. Fortunately, the mortality rates for pregnant women undergoing cardiac valvular surgery are similar to those of non-pregnant women, but the fetal mortality is high in those undergoing corrective cardiac surgery.[10]

Had patient X presented with acute heart failure before the planned Caesarean section, an urgent Caesarean section would be indicated to relieve aortocaval compression and eliminate the uteroplacental circulation which receives 10-20% of cardiac output. However, delivery does not guarantee haemodynamic improvement. In severe valvular lesions, cardiac decompensation may present or persist after delivery owing to autotransfusion of placental blood. Postoperative problems like bleeding and sepsis originating from wound infection could also lead to worsening heart failure after delivery.

CONCLUSION

Regional anaesthesia, specifically epidural anaesthesia, provides a safe alternative to general anaesthesia by maintaining hemodynamic stability and minimizing the risks such as acute right heart failure. Management strategies such as controlled fluid balance, judicious use of uterotonic drugs, and close monitoring of cardiac function are critical in preventing complications. This case reinforces the importance of individualized care and multidisciplinary collaboration among anaesthesiologists, obstetricians, and cardiologists in managing pregnant patients with congenital heart disease for Caesarean section.

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CASE REPORT

Use of Integra for thumb length conserving surgery for an acral melanoma in-situ

Jonathan Young, Elizabeth Ellul Gialanze`, Matthew Borg, Liam Mercieca

Background

Acral melanoma (AM) is an uncommon subtype of melanoma and has traditionally been managed with amputation of the affected digit, even in cases of in-situ disease. Such an approach may result in significant functional and psychological morbidity, particularly in elderly patients.

Methods

We report the case of an 80-year-old gentleman who presented with an AM in-situ affecting the left thumb, which had been present for over 20 years. Following refusal of amputation, a conservative surgical approach was undertaken. A degloving procedure was performed, and reconstruction was achieved using a dermal regeneration template (Integra®).

Results

Despite the technical and oncological limitations associated with conservative management, the patient achieved a functionally and aesthetically satisfactory outcome, with preservation of thumb length and function.

Conclusion

Although amputation of the affected digit has historically been recommended for AM, this case highlights that, in carefully selected patients with in-situ disease, a more conservative surgical approach may be a viable alternative. Such an approach may allow adequate disease control while preserving function and improving quality of life.

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Acral melanomas (AM) are a subtype of cutaneous malignant melanoma occurring on the hands and feet (palms, soles, fingers, toes, and nail units). As a result of their location, diagnosis is often delayed thus these tumours tend to present at a more advanced stage. Additionally, they pose a challenge for surgeons as considerations for functionality and cosmesis must be balanced with acquiring the recommended clinical surgical margins (eg 0.5cm margin for melanoma in situ, 1cm margin for invasive melanoma with Breslow thickness <1mm) since this type of melanoma is associated with higher local recurrence rates.[1]

CASE PRESENTATION

An 80-year-old gentleman presented to our dermatology department in August 2021 with a 20-year history of a slowly growing pigmented lesion over his left thumb. For many years, his pastime was developing photos and as a result he mistook the lesion to be a patch of ink thus failing to seek medical attention beforehand. He denied any bleeding, oozing or pruritus from the lesion. His past medical history was significant for hypertension on perindopril and bendroflumethiazide.

Examination revealed a pigmented macule with irregular borders roughly 2.5cm in diameter and involving the dorsal, medial and ventral aspects of the distal phalanx of the left thumb. The nail plate appeared grossly normal (**Figure 1**). Dermoscopic findings were highly suggestive of malignant melanoma with asymmetric colour and structure as well as an irregular, thickened pigment network. Axillary lymph nodes were not palpable. Two 3mm punch biopsies were taken; one from the medial edge of the distal phalanx and another from the pulp of the left thumb, both of which showed a melanoma in-situ, with no dermal invasion.

The management options offered to the patient at this point included wide local excision or possibly off-label use of topical imiquimod. Topical imiquimod is most often used for select lentigo malignas, usually located on the head or neck area, where surgery is deemed inappropriate, and would not be a first line treatment of acral lesions in otherwise healthy patients, due to poorer clearance rates.[2] The patient was therefore referred to plastic surgery to discuss the various surgical treatment and reconstructive options.

For early acral melanoma located on the digits, wide local excisions have been shown to have a similar prognosis compared to amputations,[3] so given the functional and aesthetic implications of a thumb amputation, the patient was offered a "degloving procedure" followed by reconstruction with a dermal regeneration template (Integra®), with consideration to proceeding to amputation if a significant invasive component was noted on the final histology report. Other reconstruction options such as abdominal or groin flaps were deemed unsuitable due to the possibility of invasive disease present in the rest of the lesion, with a risk of seeding to donor sites.

The patient categorically refused amputation and subsequently underwent local excision of the melanoma in-situ with a 0.5cm margin by the plastic surgeons, with degloving of the left thumb at the level of the interphalangeal joint and excision of the nail plate. This was followed by application of Integra®, that was doubled over at the distal aspect to provide more tissue cover. The surgery was carried out under a digital block. There were no intraoperative or postoperative complications and

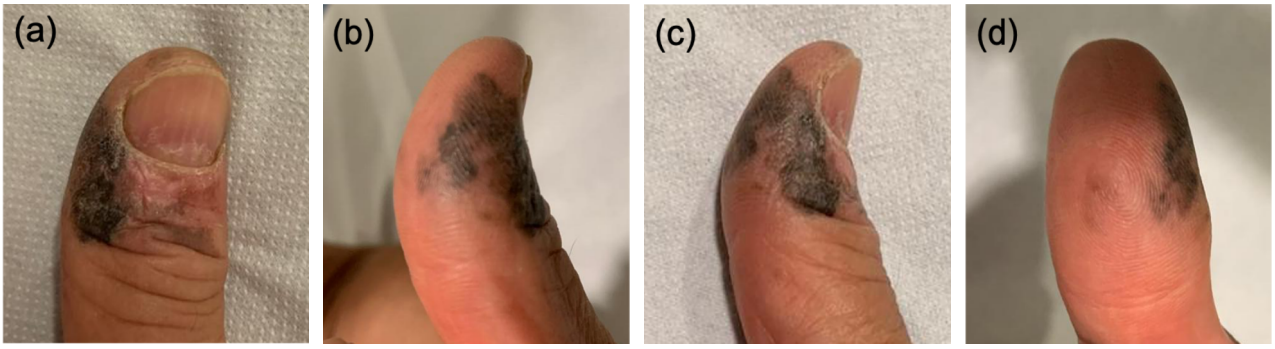


Figure 1 *(a-d) Photos of the acral melanoma in-situ at presentation.*

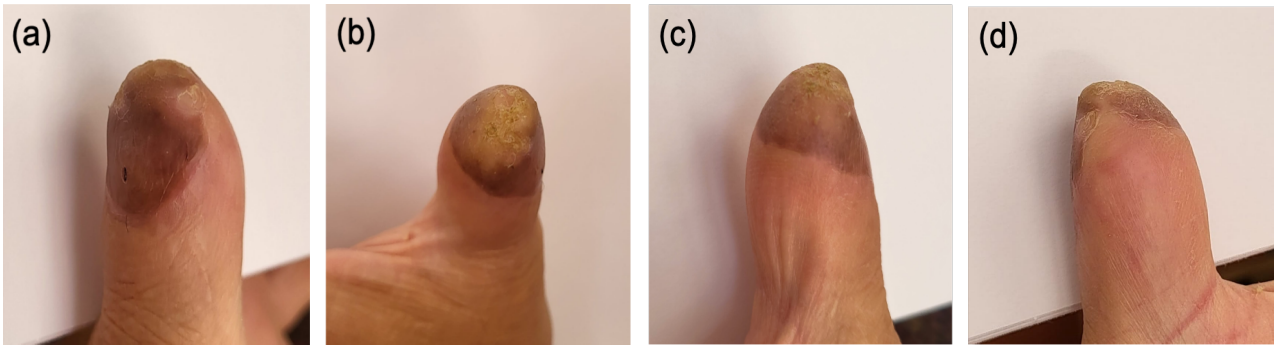


Figure 2 *(a-d) Appearance at sixteen weeks after Integra® repair.*

the patient was discharged home on a seven-day course of co-amoxiclav and pressure dressing.

In the meantime, the excised nail plate and distal portion of the left thumb were sent for histology and confirmed complete excision of the melanoma in-situ with no evidence of invasive malignancy in the nail plate and nail matrix. Moreover, a clearance of 6mm from the skin resection margin was observed. Three weeks later, a split thickness skin graft was applied over the Integra® and the patient was followed up regularly by the plastic surgeons and again at our department sixteen weeks later (**Figure 2**). He was very pleased with the results as he was able to maintain functionality of his left thumb with good cosmesis and only slight length discrepancy.

DISCUSSION

With regards to the treatment of AM located on a digit,amputation of the affected digit was the previous recommended course of action. Nevertheless, a retrospective study by Roh et al compared the incidence in local recurrence and overall survival in the group of patients undergoing amputation with those undergoing limited excision. No significant difference was found and in fact, an improved survival was noted in the latter group.[3]

With the advent of more conservative surgical techniques, the use of reconstructive measures including acellular dermal regeneration templates has increased. Integra® is a bilaminar dermal regeneration template, containing a matrix composed of glycosaminoglycans derived from shark chondroitin-6-sulfate and bovine collagen which is covered by a protective silicone sheet. The latter acts as an artificial epidermis protecting the wound from infection as well as heat and moisture loss. The inner layer can stimulate the formation of a vascularised dermis in wounds with exposed bone or tendon and those having undergone radiation thus providing a higher quality coverage compared to other reconstructive options. For this reason, the use of Integra® has been recommended in the closure of wounds secondary to traumatic degloving injuries, burns and complex cancer resections with excellent patient satisfaction and post-operative functional recovery.[4] That being said, degloving procedures in the management of acral skin cancers were seldom reported in the literature. In a case by Proietto et al, an acral melanoma in-situ was excised via "surgical degloving" and reconstruction was successfully performed by means of a radial antebrachial flap.[5]

Furthermore, several complications are associated with the use of Integra®, the commonest of which is infection. Since Integra® has no intrinsic antibacterial qualities, meticulous wound preparation and haemostasis is essential. Other complications include haematoma or seroma formation, wound breakdown and delayed immunological reactions.[6,7]

Finally, this case was intriguing since the melanoma in-situ was initially presumed to be a discolouration secondary to the photo developing liquid hence the delay in presentation and increase in size of the lesion. One of the main constituents of this liquid is hydroquinone; however, despite inhibiting melanin synthesis, it has not yet been shown to have any inhibitory or stimulatory effect on melanomas.

Based on our experience, we agree that it may be possible to consider a more conservative surgical approach to AM treatment of the digit, especially in the case of in-situ tumours. We are happy that we managed to achieve a functionally and aesthetically-satisfactory result for our patient while providing complete excision of his acral melanoma in situ.

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