Utilising features of metabolic syndrome to improve MAFLD diagnosis rates in intelligent Liver Function Testing (iLFT)

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Background

- Intelligent Liver Function Testing (iLFT), a novel, algorithm-based testing pathway, has been developed in NHS Tayside to facilitate the diagnosis of liver disease
- 30% of iLFT are returned with descriptive outcomes instead of definitive diagnoses and must progress to costly investigations and lengthy wait times
- 69% of descriptive outcomes have fatty infiltrates in their liver, consistent with Metabolic Associated Fatty Liver Disease (MAFLD)/Metabolic Associated SteatoHepatitis (MASH)
- MAFLD is closely associated with features of metabolic syndrome (glucose impairment, low-HDL cholesterol, high triglycerides, central obesity, hypertension)[1, 2]
- MAFLD is one of the leading causes of mortality from liver disease and cancer; prompt, yet cost-effective diagnosis and management are key[3]

Objectives

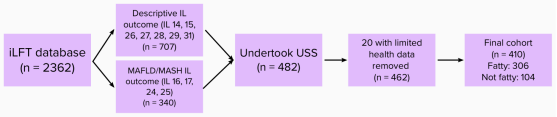
- Identify which features of metabolic syndrome are strongest predictors of fatty infiltrates in the liver
- Improve the iLFT algorithm so more patients safely receive a definitive likely diagnosis of MALFD/NASH

Results

- Current diagnostic algorithm for MAFLD that relies on clinical reporting of features of metabolic syndrome has a 45% sensitivity rate and 84% PPV
- This puts strain on downstream services that investigate the other 55% of patients
- Body Mass Index (BMI) and glucose impairment are statistically significant as strong predictors of fatty infiltrates in the liver
- iLFT algorithm can be improved by stratifying patients into three BMI categories (≤26, 27-34, ≥35) and adding HbA1c, high density lipoprotein (HDL) cholesterol, and triglycerides biochemistry analysis to certain cohorts
- The new algorithm
  - increases diagnosis rate and decreases referral to ultrasound by 18.7%
  - decreases referral to liver clinic by 8.22%
  - has a sensitivity of 95% and positive predictive value of 95%

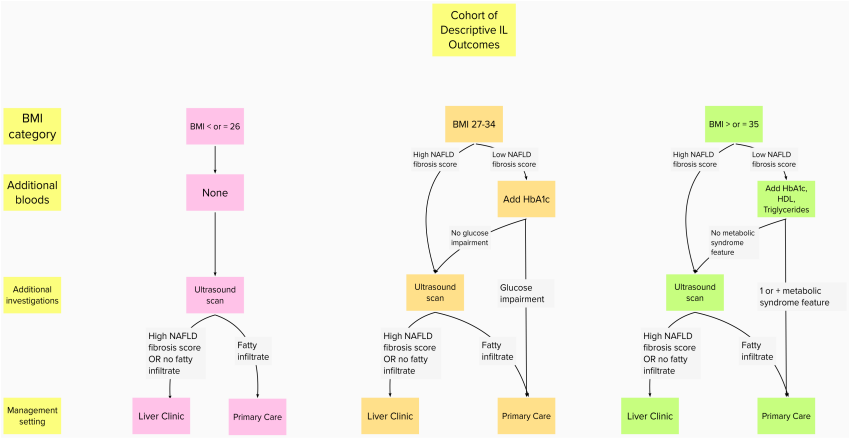
Methodology

- Patients with unknown or MAFLD-related liver blood test derangements selected from 2018-2019 iLFT database



- BMI, glucose impairment, low HDL cholesterol, high triglycerides and hypertension were entered into a binomial logistic regression analysis to predict presence of liver fat on ultrasound
- Sensitivity and positive predictive values (PPVs) were calculated for various combinations of metabolic syndrome features used to predict fatty infiltrates on ultrasound
- Metabolic syndrome features with strongest sensitivities and PPVs were applied to cohort of patients with descriptive iLFT outcomes to model an improved diagnostic algorithm with strong sensitivity and PPV

New Algorithm



Conclusions

- This project proposes an improvement to the existing diagnostic iLFT algorithm
- Features of metabolic syndrome can be used to confidently predict presence of fatty infiltrates in the liver depending on BMI
- The new algorithm can safely increase the diagnosis rate while reducing referral to ultrasound scan by 18.70% and reducing referral to liver clinic by 8.22% (95% sensitivity, 95% PPV)
- Further research with more complete datasets including waist circumference, biopsy, and specialist hepatology opinion is recommended

References

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AMR Genes Metadata for Uropathogenic Strains of E. coli and K. pneumoniae and Mapping to Reference Genomes

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Introduction

Antimicrobial Resistance (AMR) is growing into an alarming problem in the healthcare sector. As bacteria are becoming more resistant to widely used antibiotics and these in turn are becoming ineffective, researchers in bioinformatic and genomics are gaining recognition in this rapidly evolving field.

The purpose of this research project was to gain a deeper understanding in the field of bioinformatics and genomics, in order to carry out most of the tasks an understanding of programming languages such as Unix and R were needed. The main focus of this project was to annotate AMR genes of Uropathogenic strains of E. coli and K. pneumoniae, this data was provided by collection clinics in both Kenya and Uganda were urine samples from patients with UTIs were taken.

Many different aspects of genomics were investigated throughout the project, for simplicity it has been divided into three main tasks. The first task was to create a novel genome assembly from raw sequence reads (See Figure 1). The second task was to create metadata of AMR genes for every sample (See Figure 2). The third task was to map the sequences from the clinics to a reference sequence and calculate the coverage and gene copy number (See Figure 3).

Methods

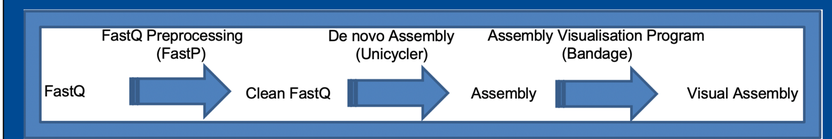


Figure 1 shows the method used to make a novel assembly with a raw sequence read in the form of a FastQ file. After the assembly had been made it could be visualised with an assembly visualisation program (See Figure 5)

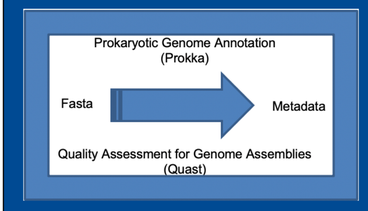


Figure 2 shows how the AMR gene metadata was created from the sequence reads in the Fasta file format. A few AMR genes were selected for each antibiotic class, a database with all AMR genes was then crunched with the sequence reads from the clinics and finally a genome annotation program was used to find the AMR genes.

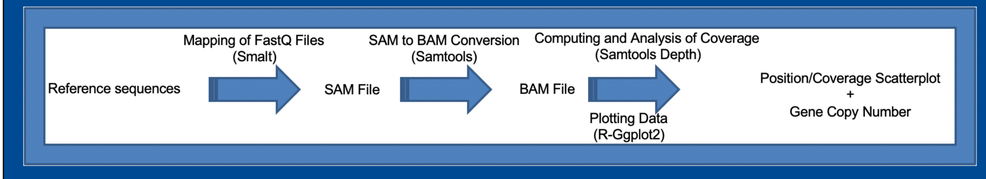


Figure 3 shows how the reference sequences in the FastQ format acquired from the clinics were mapped to a reference sequence and were analysed to give a position/coverage scatterplot and a gene copy number for each AMR gene (See Graph 1)

Results

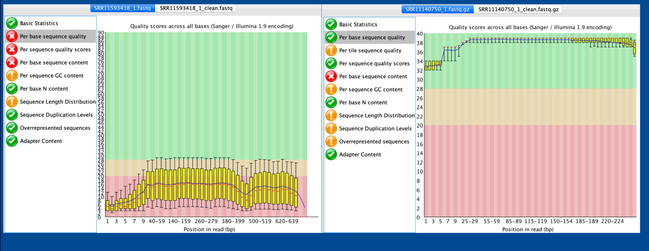


Figure 4 shows two FastQC reports of two different samples used in this project. FastQC is a platform which highlights if there are any problems with your data that you should be aware of before carrying out a more in depth analysis of the read sequences.

On the left you can see that the quality score given for the read is quite low, this means that it is going to be harder to work with this sequence as it will be more inaccurate. However, the screenshot on the right shows a high quality score which suggests that the sequence is accurate and is suitable for further analysis.

FastQC is used before FastQ Preprocessing (See Figure 1), poor sequence reads can be put through a processing program which will format it and improve the quality of the read.

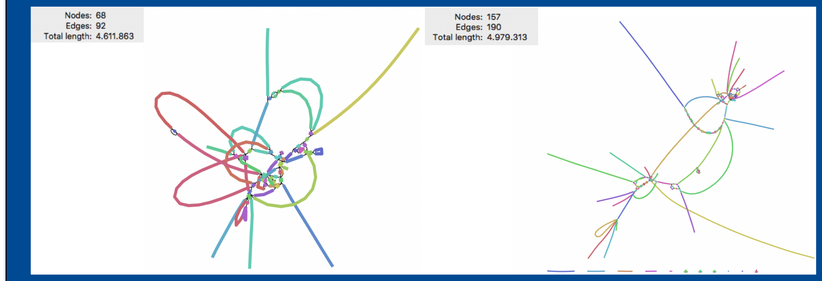


Figure 5 shows two de novo assemblies from two different samples of E. coli collected from the clinic in Kenya. The program bandage allows for the visualisation of assemblies but it will also show the total length of the genome, the number of nodes and edges.

A perfect assembly would have no nodes and would be one continuous genome. The assembly on the left side would therefore be considered as a more reliable assembly as it has fewer nodes than the assembly on the right.

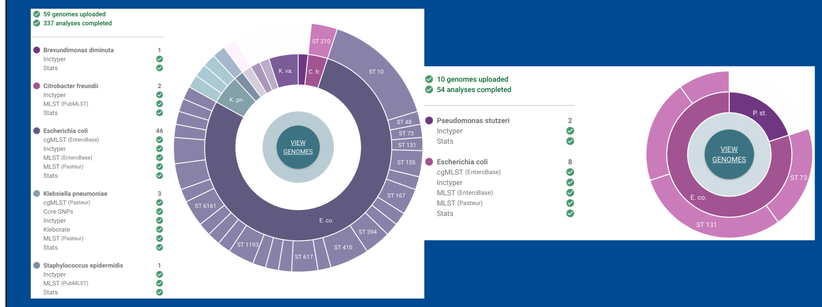
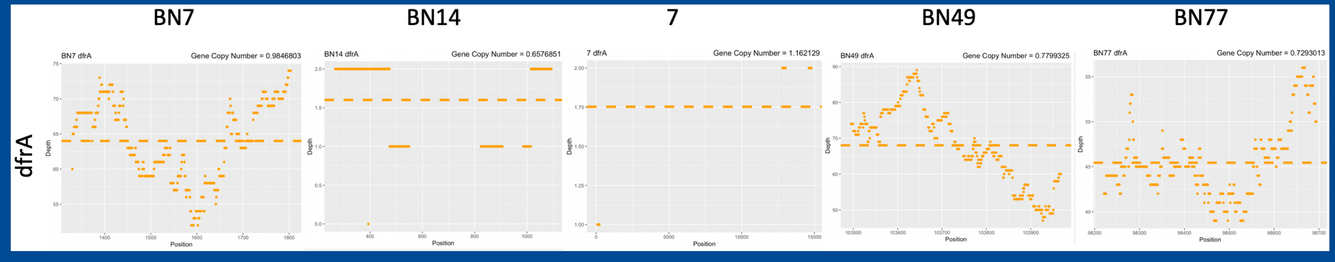


Figure 6 shows two reports from the taxonomy prediction program Pathogenwatch and it is the first step in creating the AMR gene metadata (See Figure 2). A taxonomy prediction program takes the sequence reads in Fasta format and analyses what organism it is. This is important as the AMR metadata is only for E. coli and K. pneumoniae.

The report on the left corresponds to the samples collected from the clinic in Uganda. The majority of the samples here are E. coli and K. pneumoniae but there are also some other pathogens like S. epidermidis.

The report on the right shows the analysis for the samples collected from the clinic in Kenya. The majority of the samples here belong to E. coli but there are two samples which correspond to P. stutzeri.



Graph 1 shows the dfrA gene (which conveys resistance to antibiotics of the folate inhibitor pathway class) from the Ugandan and Kenyan E. coli sequence reads mapped onto the reference sequences. The base position is plotted against the depth/coverage. The dashed line represents the mean coverage/depth and the gene copy number is noted on the top right of the scatterplot. The gene copy number for all the dfrA genes above can be rounded to 1 giving a result of one copy of the gene within the genome of the organism.

Conclusion

After creating the metadata for both Kenyan and Ugandan E. coli and K. pneumoniae I could see that most of the organisms had one or more genes that provided them with resistance to nearly all main classes of antibiotics. By taking part in this project I have been able to appreciate how big of a problem the increasing resistance to common antibiotics is.

For me one of the most important things I have learnt is how important collaboration is within people from the same and other fields. Most of the data used for this project had been shared from other parts of the world for me to personally analyse, without this help I probably would have not been able to complete this research project.

A subgroup analysis on the use of acyclovir and/or prednisolone for the early treatment of Bell's palsy:

How does age affect the severity of Bell's palsy cases, at presentation and through the extent of recovery, at three and nine months?

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ABSTRACT

BACKGROUND Corticosteroids have recently demonstrated to be of greater benefit for patients with Bell's palsy, when compared to the traditional antiviral administered, acyclovir. However, the effect of age on the prognosis of Bell's palsy and effectiveness of these therapeutic options is unknown.

METHODS We based this study on the double-blind, randomized BELLS study, using the data set provided by the authors. Patients were assigned one of four treatments - prednisolone and placebo, acyclovir and placebo, acyclovir and prednisolone, or placebo and placebo; and monitored at three and nine months, using the House-Brackmann scale. By secondary analysis of this data, the 496 patients, aged 16-90 years, were sorted by score at presentation, three, and nine months.

RESULTS Patients were found to have different recovery rates and different responses to treatment. Those aged 16-44 and 75-90 years recovered better than those aged 45-74 years. Additionally, from 60 years onwards the effectiveness of prednisolone plus placebo, the treatment preferred in the younger subjects, declines – in patients within the 60-90 years age band combination therapy of acyclovir plus prednisolone proved to offer a fuller recovery.

CONCLUSION Patients aged 16-44 and 75-90 years display a fuller and faster recovery through treatment. From 60 years onwards, the effectiveness of prednisolone alone declines, and combination therapy with acyclovir is a better therapeutic option with these patients.

OBJECTIVES

PRIMARY Discern a relationship of importance between age and severity of Bell's palsy at both onset and through treatment

SECONDARY Determine the role of age on the efficacy of each treatment

METHODS

PATIENTS

Patient data was obtained from the BELLS study authors, fully anonymized and reduced to the patient's ID, sex, age, treatment received, and House-Brackmann score at 0, 3, and 9 months

496 patients were used in the study (253 male, 243 female)

Age range from 16 to 90 years (mean 44±16.4 years)

Subdivision into age bands (years): 16-29, 30-44, 45-59, 60-74, 75-90

STATISTICAL ANALYSIS

Data analysis conducted using Excel

The House-Brackmann score is used for the evaluation of facial nerve paralysis, and determined by measurement of both the upwards movement of the midportion of the top of the eyebrow, as well as the outwards movement of the mouth. Based on functional impairment, the scale ranges from 1 (normal facial function) to 6 (severely impaired facial function) (Table 1).

Score	Impairment
1	Normal
2	Mild dysfunction (slight weakness, normal symmetry at rest)
3	Moderate dysfunction (obvious but not disfiguring weakness with synkinesis, normal symmetry at rest) Complete eye closure with maximal effort, good forehead movement
4	Moderately severe eye dysfunction (obvious and disfiguring asymmetry, significant synkinesis) Incomplete eye closure, moderate forehead movement
5	Severe dysfunction (barely perceptible motion)
6	Total paralysis (no movement)

Table 1 - House-Brackmann Facial Paralysis Scale[7]

RESULTS

Characteristic	Age Band – years					Total
Sex – no (%)	16 - 29	30 - 44	45 - 59	60 - 74	75 - 90	
Male	59 (53.6)	82 (53.2)	67 (47.5)	36 (50.7)	9 (45)	253
Female	51 (46.4)	72 (46.8)	74 (52.5)	35 (49.3)	11 (55)	243
Total	110	154	141	71	20	497
Score on House-Brackmann Scale – no (%)						
1	8 (7.2)	11 (7.1)	11 (7.8)	3 (4.2)	1 (5)	34 (6.8)
2	15 (13.6)	15 (9.7)	7 (5)	8 (11.3)	0 (0)	45 (9.1)
3	33 (30)	43 (27.9)	43 (30.5)	12 (16.9)	7 (35)	138 (27.8)
4	31 (28.2)	48 (31.2)	38 (27)	19 (26.8)	2 (10)	138 (27.8)
5	17 (15.5)	31 (20.1)	25 (17.7)	21 (29.6)	7 (35)	101 (20.3)
6	1 (0.9)	5 (3.2)	13 (9.2)	6 (8.5)	3 (15)	28 (5.6)
Treatment – no (%)						
OP	29 (26.4)	47 (30.5)	31 (22)	15 (21.1)	5 (25)	127
OO	29 (26.4)	36 (23.4)	32 (22.7)	23 (32.4)	2 (10)	122
AP	29 (26.4)	36 (23.4)	37 (26.2)	16 (22.5)	6 (30)	124
AO	23 (21)	35 (22.7)	41 (29.1)	17 (23.9)	7 (35)	123

Table 2 - Baseline Characteristics of Patients

IMPACT OF AGE ON PRESENTING SEVERITY AND TREATMENT OUTCOME

As age increases, the proportion of patients attaining a full recovery at 3 months decreases (figure 1).

Age Band	0M	3M	9M
16-29	0.0762	0.8679	0.9182
30-44	0.0719	0.7919	0.9286
45-59	0.0803	0.6714	0.8085
60-74	0.0435	0.5775	0.8451
75-90	0.0500	0.6000	0.9500
P	-	<0.001	0.0105

Table 3 - Proportion of patients with full recovery, through treatment, in each age band

We found an increasing severity amongst age bands at presentation, illustrated by the percentage of patients in each age band presenting with a House-Brackmann score between 3 and 6, indicating moderate to severe paralysis:

- 74.6% patients of those aged 16-29 years
- 81.8%-84.4% of patients in groups aged 30-44, 45-59, and 60-74 years
- 95% of patients in age band 75-90 years

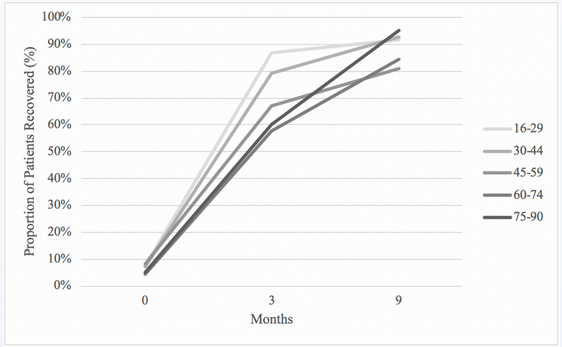

Figure 1 - Percentage of patients with full recovery, through treatment, in each age band

IMPACT OF AGE ON EFFICACY OF THERAPEUTIC OPTIONS

Treatment	Age Band				
	16-29	30-44	45-59	60-74	75-90
OP	0.9310	0.8511	0.8710	0.6000	0.8000
OO	0.6897	0.7222	0.5938	0.4783	0.5000
AP	0.8966	0.7778	0.7297	0.6875	1.0000
AO	0.8261	0.6857	0.5122	0.5882	0.1429
Total	0.8679	0.7919	0.6714	0.5775	0.6000

Table 4 - Proportion of patients fully recovered at three months, by treatment (P<0.001) (OP = placebo plus prednisolone; OO = placebo plus placebo; AP = acyclovir plus prednisolone; AO = acyclovir plus placebo)

At 3 months, the most effective treatments proved to be:

- Prednisolone alone for those aged 16-59 years
- Combination therapy of prednisolone plus acyclovir from 60 years onwards

Recovery rate at 3 months (Table 4), with the most effective treatment within the age band:

- 93% of patients aged 16-29 years, receiving OP, fully recovered
- 85-87% patients aged 30-59 years, with OP, fully recovered
- 68.8% patients, receiving AP, aged 60-74 years
- 100% of patients aged 75-90 years receiving AP fully recovered

Treatment	Age Band				
	16-29	30-44	45-59	60-74	75-90
OP	1.0000	0.9574	1.0000	0.8000	1.0000
OO	0.8276	0.9722	0.7813	0.7826	1.0000
AP	1.0000	0.8889	0.8919	0.9375	1.0000
AO	0.8261	0.8857	0.6098	0.8824	0.8571
Total	0.9182	0.9286	0.8085	0.8451	0.9500

Table 5 - Proportion of patients fully recovered at nine months, by treatment (P=0.0105) (OP = placebo plus prednisolone; OO = placebo plus placebo; AP = acyclovir plus prednisolone; AO = acyclovir plus placebo)

At 9 months, the effectiveness of treatments was maintained, in each age band (Table 4).

In patients above the age of 60 years, AP provided the best therapeutic outcome (P = 0.03388), whilst OP reflected no major improvement in the recovery of these patients (P = 0.07731).

Recovery rate at 9 months (Table 5), with the most effective treatment within the age band:

- 100% of patients aged 16-29 years, receiving OP, fully recovered
- 95-100% patients aged 30-59 years, with OP, fully recovered
- 93.8% patients, receiving AP, aged 60-74 years

CONCLUSIONS

Patients aged 16-44 years and 75-90 years will typically have a better prognosis for recovery of Bell's palsy.

Additionally, we demonstrated that the use of acyclovir with prednisolone should be the treatment of choice for patients aged 60 years or more.