Project Report Summary

QIBA 2014 Project Deliverables:
1. Literature review. Rank order list of potential confounders in order of perceived clinical importance.
2. Clinical study. Multivariate analysis of the effect of steatosis and inflammation on shear wave speed as determined by SWE.
   • Target recruitment 65-85.

QIBA 2014 Summary Report:
1. Literature review:
   • Comprehensive literature search comprising review of ~1,548 abstracts screened to identify all SWS papers and detailed review of ~ 102 SWS research papers completed.
   • Confounders identified (see detailed report below – pages 2-5).
   • Comprehensive report concerning confounders presented to QIBA US group and shared.
2. Clinical study
   • Target enrollment exceeded, with 242 subjects enrolled against a target of 85.
   • Single-reader pathology completed for all 242 subjects.
   • Statistical analysis including multivariate analysis completed (see detailed report attached – Pages 6-12).
3. Work complete additional to listed deliverables:
   • Completion of elastography eCRF, hosted at REDCap and publicly available.
   • Completion of pathology eCRF, hosted at REDCap and publicly available.
   • UPICT guidelines.
   • RSNA 2014 QIBA US poster: “RSNA/QIBA: Variability Sources and Potential Mitigation Strategies in Shear Wave Elastography for the Staging of Liver Fibrosis.”

New Knowledge:
• Standard deviation within elastography values for each subject can be used as a quality factor.
• Standard deviation <20 appears to be the optimal quality factor in our dataset.
• Confirmation - Steatosis has no effect on SWS speed.
• Confirmation - Inflammation is an independent predictor of SWS.

Future Plans:
• Manuscripts:
  ▪ Summary of Literature Review – “Technical and Clinical Confounders in Elastography”.
  ▪ Original research based on clinical study –
    1. Quality metrics for elastography.
    2. Establishing clinical confounders in elastography and correcting for their effects.

Note: Shear wave speeds have been documented as a measure of young’s modulus of elasticity in kilopascals (kPa) throughout the report, and shear wave elastography (SWE) is the term used to represent the application of shear wave speeds to estimate liver fibrosis.
Detailed Report: Deliverable 1

Rank order list of potential confounders in order of perceived clinical importance:

- A literature review of 1,548 publications after a broad search was performed.
- 102 SWS and ARFI papers that studied confounders were identified.
- A Dropbox folder shared with the “QIBA SWS committee” contains a detailed Excel sheet reporting findings of these 102 papers.
- All potential confounders in the literature are:

<table>
<thead>
<tr>
<th>Technical Factors</th>
<th>Imaging factors</th>
<th>Clinical Factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Depth</td>
<td>1. Fasting/Meals</td>
<td>1. Steatosis</td>
</tr>
<tr>
<td>2. Probe</td>
<td>2. BMI</td>
<td>2. Inflammation</td>
</tr>
<tr>
<td>3. Patient position</td>
<td>3. Right heart insufficiency</td>
<td></td>
</tr>
<tr>
<td>4. Patient breathing</td>
<td>4. Cholestasis</td>
<td></td>
</tr>
<tr>
<td>5. Lobe of liver</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Reproducibility</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Each parameter is described in detail in the shared Dropbox folder, with sub-folders containing research papers relevant to each parameter.
- Full-text of manuscripts that study these factors are available in these sub-folders.

Steatosis and inflammation are the most common pathological processes occurring in conjunction with fibrosis in chronic liver disease. A detailed literature review of these two factors found that:

- **Steatosis**: Extensive evidence shows that steatosis does not affect shear wave speed.
  - Relevant references are listed in **Annexure 1**.
- **Inflammation**: Extensive evidence inflammation increases shear wave speed, with some disagreement in the literature.
  - Inflammation influences SWS values (**References - Annexure 2**)
  - No correlation of SWS with inflammation (**References - Annexure 3**)

Annexure 1
(Steatosis has no effect on SWS)

References:


Annexure 2
(Inflammation affects SWS)

References:


Annexure 3
(Inflammation does not affect SWS)


Detailed Report: Deliverable 2
Multivariate analysis of the effect of steatosis and inflammation on shear wave speed.

Study Design:
• Consecutive patients scheduled for a liver biopsy in our hospital underwent elastography prior to the liver biopsy.
• 10 elastography values were obtained from the right upper lobe via an intercostal approach.
• Liver biopsies were read by a single sub-specialist pathologist (as per the detailed Pathology case report form).

Study Results:
Recruitment: 242
Pathology Review: Completed for 242 cases

Results:
1. Summary and evaluation of best statistical method to calculate representative value of liver stiffness. (Annexure 1)
2. Fibrosis (Annexure 2)
   • Shear wave speed (SWS) represented by a young’s modulus of elasticity in kPa correlate with fibrosis stage \( p<0.001, r=0.498 \)
   • Receiver-operating characteristic (ROC) curves showed an area under the curve (AUROC) of 0.84 (0.78-0.91).
3. Steatosis (Annexure 3)
   • Steatosis does not correlate with SWS \( (p = 0.229, r = -0.086) \)
4. Inflammation (Annexure 4)
   • SWS correlates with the total activity score as assessed by the METAVIR scoring system \( (p <0.001, r = 0.398) \).
5. Multivariate analysis (Annexure 5)
   • Inflammation and fibrosis are independent predictors of SWS \( (p<0.001) \) while steatosis is not a significant predictor.
   • The effect of inflammation on fibrosis differs in each stage of fibrosis. Subgroup analysis to follow.
Annexure 1 – Statistical Analysis

Total number of patients recruited = 242
Pathology available = 242

Excluded = 28
  Allografts = 24
  Granulomatous = 1
  Not enough images/very poor images = 3

Total number of cases included in analysis = 214

Statistical method used to calculate Median SWE value as an estimate of Young’s modulus in kPa.
Different statistical methods of calculating the SWE value or selecting patients based on the obtained SWE values were tested and are listed below:

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. All</td>
<td>All cases without exclusion of any SWE values</td>
</tr>
<tr>
<td>2. IQR</td>
<td>Median SWE value is calculated after dropping off values below the 25th percentile and above the 25th percentile.</td>
</tr>
<tr>
<td>3. TM5</td>
<td>Mean SWE value is calculated after dropping off the lower 5% and highest 5% values.</td>
</tr>
<tr>
<td>4. TM10</td>
<td>Mean SWE value is calculated after dropping off the lower 5% and highest 10% values.</td>
</tr>
<tr>
<td>5. TM25</td>
<td>Mean SWE value is calculated after dropping off the lower 5% and highest 25% values.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Selecting subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>6. SD&lt;20</td>
</tr>
<tr>
<td>7. SD&lt;10</td>
</tr>
<tr>
<td>8. SD&lt;5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>IQR</th>
<th>TM5</th>
<th>TM10</th>
<th>TM25</th>
<th>SD&lt;20</th>
<th>SD&lt;10</th>
<th>SD&lt;5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>214</td>
<td>214</td>
<td>214</td>
<td>214</td>
<td>214</td>
<td>195</td>
<td>179</td>
<td>160</td>
</tr>
<tr>
<td>F0</td>
<td>73</td>
<td>73</td>
<td>73</td>
<td>73</td>
<td>73</td>
<td>63</td>
<td>61</td>
<td>58</td>
</tr>
<tr>
<td>F1</td>
<td>88</td>
<td>88</td>
<td>88</td>
<td>88</td>
<td>88</td>
<td>81</td>
<td>75</td>
<td>67</td>
</tr>
<tr>
<td>F2</td>
<td>26</td>
<td>26</td>
<td>26</td>
<td>26</td>
<td>26</td>
<td>26</td>
<td>23</td>
<td>20</td>
</tr>
<tr>
<td>F3</td>
<td>21</td>
<td>21</td>
<td>21</td>
<td>21</td>
<td>21</td>
<td>20</td>
<td>17</td>
<td>14</td>
</tr>
<tr>
<td>F4</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>SC</td>
<td>0.41</td>
<td>0.41</td>
<td>0.368</td>
<td>0.368</td>
<td>0.397</td>
<td><strong>0.498</strong></td>
<td><strong>0.456</strong></td>
<td>0.431</td>
</tr>
<tr>
<td>AUROC</td>
<td>0.79</td>
<td>0.79</td>
<td>0.77</td>
<td>0.77</td>
<td>0.78</td>
<td><strong>0.84</strong></td>
<td><strong>0.855</strong></td>
<td>0.86</td>
</tr>
</tbody>
</table>

- Statistical methods used to obtain the representative SWE value without excluding any cases did not show better results.
- However, selecting cases that have a relatively uniform SWE value i.e. standard deviation less than 20 and standard deviation less than 10 did show a significant improvement in accuracy. Hence cases that had a standard deviation <20 amongst all obtained SWE values, were selected for analysis. Median SWE values were used.
Annexure 2 - Fibrosis

All Measurements:

Figure 1: SWE values as plotted against Fibrosis stages (F0-F4) for all enrolled cases (n=214)

Selecting cases with standard deviation less than 20.

Figure 2: SWE values as plotted against Fibrosis stages (F0-F4) for selective cases when standard deviation in obtained SWE values less than 20 (n=195)
Values for each fibrosis grade for selective patients (n=195)

<table>
<thead>
<tr>
<th>Fibrosis</th>
<th>F0</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median</td>
<td>6.65</td>
<td>7.65</td>
<td>9.85</td>
<td>13.88</td>
<td>31.9</td>
</tr>
<tr>
<td>Mean</td>
<td>7.18</td>
<td>8.49</td>
<td>10.83</td>
<td>15.86</td>
<td>30.95</td>
</tr>
<tr>
<td>95%CI</td>
<td>6.61-7.75</td>
<td>7.44-9.55</td>
<td>8.96-12.71</td>
<td>12.02-19.69</td>
<td>13.94-47.96</td>
</tr>
<tr>
<td>Std.Dev</td>
<td>2.26</td>
<td>4.78</td>
<td>4.65</td>
<td>8.20</td>
<td>13.70</td>
</tr>
<tr>
<td>Min.</td>
<td>2.6</td>
<td>1.80</td>
<td>5.90</td>
<td>5.20</td>
<td>13.45</td>
</tr>
<tr>
<td>Max.</td>
<td>15.70</td>
<td>31.35</td>
<td>28.30</td>
<td>40.15</td>
<td>50.70</td>
</tr>
</tbody>
</table>

Area under the ROC curve

- Area under the ROC curve to differentiate significant fibrosis (F2-F4) from lesser degrees of fibrosis (F0-F1) is 0.84 (0.78-0.91)

Figure 3: Receiver operating characteristic curve that differentiates higher degrees of fibrosis (F2-F4) from lesser degrees of fibrosis (F0-F1).
Annexure 3 - Steatosis

<table>
<thead>
<tr>
<th>Steatosis Stage</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>70</td>
</tr>
<tr>
<td>1</td>
<td>39</td>
</tr>
<tr>
<td>2</td>
<td>31</td>
</tr>
<tr>
<td>3</td>
<td>40</td>
</tr>
<tr>
<td>4</td>
<td>15</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>195</strong></td>
</tr>
</tbody>
</table>

Table: Number of subjects in each stage of steatosis

Box and whisker plot that charts median SWE values for each steatosis stage within each fibrosis stage. Steatosis stages are color coded as per the index on the top-right.

Spearman’s Correlation with steatosis \( r = -0.086 \) \( p = 0.229 \)
Annexure 4 - Inflammation

<table>
<thead>
<tr>
<th>TAS Score</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>70</td>
</tr>
<tr>
<td>1</td>
<td>91</td>
</tr>
<tr>
<td>2</td>
<td>27</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>195</td>
</tr>
</tbody>
</table>

Table: Total activity score (TAS) On METAVIR staging system

Box and whisker plot that charts median SWE values for each Total activity score that represents inflammation on the METAVIR system.

Box and whisker plot that charts median SWE values for each inflammation stage within each fibrosis stage. Total activity scores are color coded as per the index on the top-right.

Spearman’s correlation with the total activity score p<0.001, r= 0.398
Annexure 5
Multivariate regression analysis to study the effect of steatosis and inflammation on SWE in the presence of fibrosis

Tests of Model Effects

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wald Chi-Square</td>
<td>df</td>
<td>Sig.</td>
</tr>
<tr>
<td>(Intercept)</td>
<td>591.943</td>
<td>1</td>
<td>.000</td>
</tr>
<tr>
<td>MET_TAS</td>
<td>32.659</td>
<td>3</td>
<td>.000</td>
</tr>
<tr>
<td>MET_Fibrosis</td>
<td>144.164</td>
<td>4</td>
<td>.000</td>
</tr>
<tr>
<td>MET_Steatosis</td>
<td>4.344</td>
<td>4</td>
<td>.361</td>
</tr>
<tr>
<td>MET_Fibrosis * MET_TAS</td>
<td>39.081</td>
<td>10</td>
<td>.000</td>
</tr>
</tbody>
</table>

Dependent Variable: Median SWE (kPa)
Model: (Intercept), MET_TAS, MET_Fibrosis, MET_Steatosis, MET_Fibrosis * MET_TAS

1. Inflammation and fibrosis are independent predictors of SWE (p<0.001) while steatosis is not a significant factor.
2. The effect of inflammation on fibrosis differs in each stage of fibrosis.