HEPATITIS C RESISTANCE TESTING
MACHAR COLONY, KARACHI, 2018
Introduction

Médecins Sans Frontières (MSF) is an international, independent, medical humanitarian organisation that delivers emergency aid to people affected by conflict, epidemics, natural disasters, and exclusion from healthcare. We offer assistance to people based on need and irrespective of race, religion, gender, or political affiliation.

MSF was founded in Paris, France in 1971. Today, MSF is a worldwide movement of 24 associations. MSF International, which binds these associations together, is based in Geneva, Switzerland. We provide medical care to help people survive catastrophic situations, where communities and health structures may be overwhelmed. Our actions are guided by medical ethics and the principles of neutrality and impartiality. We do not take sides but seek to bring assistance to those who need it most urgently. In situations of conflict, we do not accept funds from governments or other parties who are directly involved.

MSF is committed to addressing the suffering people endure and the obstacles encountered in providing assistance. We are constantly seeking to improve the quality, relevance and extent of our assistance, and we are dedicated to innovation. When we witness violations of international humanitarian law or neglected crises, MSF may speak out about this. In more than 70 countries, Médecins Sans Frontières provides medical humanitarian assistance to save lives and ease the suffering of people in crisis. Its work is carried out by thousands of health professionals, logistical and administrative staff, the vast majority of whom come from the countries where the organisation is providing medical assistance.

MSF first began working in Pakistan in 1986 and now provides urgently needed medical care to people in Balochistan, Khyber Pakhtunkhwa, and Sindh provinces. All services are provided free of cost. MSF’s activities in Pakistan are funded solely by donations from individuals from all around the world, with no institutional or government contributions.

MSF and Hepatitis C

An estimated 71 million people are infected with the hepatitis C virus globally – double the number living with HIV.¹ Hepatitis C is found worldwide; however, the vast majority of people with the disease live in developing countries: China, Pakistan, India, Egypt and Indonesia are particularly affected. While hepatitis C can be cured, millions have no access to treatment, some because they have never been tested and do not know they need it and some because they cannot afford it. MSF runs 11 medical projects worldwide providing screening and curative services for Hepatitis C, including vertical programmes as well as programmes addressing HIV co-infection. Since April 2014, tens of thousands of people have been screened, with more than 10,000 testing positive and around 5,000 started on treatment. Of those who have completed treatment to date, the overall cure rate measured by sustained virological response at 12 weeks (SVR12) is 94.9 per cent.²
Hepatitis C in Pakistan

The prevalence of chronic HCV infection (CHC) in adults in Pakistan is estimated at 5.8 - 6.8%.³,⁴ (range 1.4 - 8.7%) HCV transmission appears to be primarily driven by healthcare-related exposure, such as therapeutic injections, intravenous infusions, transfusion of poorly screened blood, and lack of sterilisation of medical equipment.⁵ With an estimated 7.1 million CHC patients (range 1.7-10.5 million), Pakistan has the second highest viraemic population after China.⁶ Genotype 3 (69.1%) is the most prevalent genotype in Pakistan.⁷

MSF Hepatitis C Project, Karachi

In 2015 MSF established a hepatitis C project in a low socio-economic area of Karachi, integrated with its own established Primary Health Centre (PHC) in Machar Colony. In two and a half years, approximately 12,000 patients presenting to the PHC have been screened according to WHO HCV-screening criteria, and rapid diagnostic testing showed 30% sero-positivity. Conversion to chronic viraemia remains at around 60-65% of those testing positive on the antibody test. All testing positive are eligible for confirmatory testing and treatment; priority and length of treatment with Direct Acting Antivirals (DAAs) have been determined on the basis of fibrosis using APRI score. Patients are followed for treatment duration and tested at 12 weeks post-treatment for sustained virological response (SVR12). Those still showing viraemia at SVR12 are offered further testing and treatment. Of the 2,285 patients initiated on treatment, 1,633 have completed treatment so far. Of these patients, 12% have been lost to follow-up, and 3% have failed treatment and are intended to be re-treated. The rest have been marked cured⁸.

Resistance testing

DAAs have high efficacy as compared to interferon-based therapy, with SVR expected greater than 90%. However, they fail to eliminate the infection in 1-6% of patients.⁹,¹⁰ Although the majority of failures are due to relapse after completion of treatment course, presence of Resistance Associated Substitutions (RAS) are a significant cause of failure.¹¹ RAS affects gene sequencing of those proteins which are involved in protein synthesis. These are the sequences (NS5A, NS5B and NS3A) attacked by DAAs.

Due to the unavailability of resistance testing within Pakistan, the MSF Karachi project decided to send dried blood spot samples from the first ten treatment failures were sent to the Laboratoire de Virologie, Hôpitaux Universitaires de Genève (HUG) for resistance sequencing and drug sensitivity profile, and nine yielded results.
Table 1, below, shows key patient information from nine patients treated at the MSF Hepatitis C Clinic in Machar Colony, Karachi, Pakistan.

### Table 1 - Pre- and Post-Treatment Viral Load in patients failing to achieve SVR12

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Age/ Sex</th>
<th>Tx History</th>
<th>APRI</th>
<th>Pre-Tx Viral Load</th>
<th>Tx Regime</th>
<th>Post-Tx Viral Load</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>36F</td>
<td>Peg-Inf, DAA</td>
<td>3.2</td>
<td>405,553</td>
<td>SOF+DAC 24</td>
<td>34,600</td>
</tr>
<tr>
<td>2</td>
<td>50F</td>
<td>NAÏVE</td>
<td>3.36</td>
<td>56,016</td>
<td>SOF+DAC+RIB 12</td>
<td>503,000</td>
</tr>
<tr>
<td>3</td>
<td>40F</td>
<td>NAÏVE</td>
<td>1.51</td>
<td>49,886</td>
<td>SOF+DAC 12</td>
<td>111,000</td>
</tr>
<tr>
<td>4</td>
<td>35M</td>
<td>NAÏVE</td>
<td>1.35</td>
<td>174,658</td>
<td>SOF+DAC 12</td>
<td>440,000</td>
</tr>
<tr>
<td>5</td>
<td>51M</td>
<td>NAÏVE</td>
<td>0.82</td>
<td>568,628</td>
<td>SOF+DAC 12</td>
<td>1,230,000</td>
</tr>
<tr>
<td>6</td>
<td>35F</td>
<td>NAÏVE</td>
<td>0.93</td>
<td>208,530</td>
<td>SOF+DAC 12</td>
<td>408,000</td>
</tr>
<tr>
<td>7</td>
<td>45F</td>
<td>NAÏVE</td>
<td>0.99</td>
<td>517,232</td>
<td>SOF+DAC 12</td>
<td>139,000</td>
</tr>
<tr>
<td>8</td>
<td>45F</td>
<td>NAÏVE</td>
<td>0.56</td>
<td>2,107,460</td>
<td>SOF+DAC 12</td>
<td>1,600,000</td>
</tr>
<tr>
<td>9</td>
<td>45M</td>
<td>NAÏVE</td>
<td>3.0</td>
<td>57,129</td>
<td>SOF+DAC 24</td>
<td>7,350</td>
</tr>
</tbody>
</table>

**Colour Legend**
- ↑ Viral Load
- ↓ Viral Load

### Results

From the nine Dried Blood Spot samples that yielded results for NS3 and NS5A resistance testing, it was established that failures or non-responders to DAAs were not due to under-treatment or patient adherence, but rather due to resistance.

### Table 2 - Resistance pattern of isolated HCV virus against common NS5A inhibitors

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Tx History</th>
<th>Tx Regime</th>
<th>Genotype</th>
<th>Mutation</th>
<th>DACLATASVIR Resistance</th>
<th>VELPATASVIR Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Peg-Inf, DAA</td>
<td>SOF+DAC 24</td>
<td>3a</td>
<td>93H</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>2</td>
<td>NAÏVE</td>
<td>SOF+DAC+RIB 12</td>
<td>3b</td>
<td>31M</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>3</td>
<td>NAÏVE</td>
<td>SOF+DAC 12</td>
<td>3b</td>
<td>31M</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>4</td>
<td>NAÏVE</td>
<td>SOF+DAC 12</td>
<td>3b</td>
<td>31M</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>5</td>
<td>NAÏVE</td>
<td>SOF+DAC 12</td>
<td>3b</td>
<td>31M</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>6</td>
<td>NAÏVE</td>
<td>SOF+DAC 12</td>
<td>3b</td>
<td>31M</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>7</td>
<td>NAÏVE</td>
<td>SOF+DAC 12</td>
<td>3b</td>
<td>31M</td>
<td>R</td>
<td>S</td>
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<tr>
<td>8</td>
<td>NAÏVE</td>
<td>SOF+DAC 12</td>
<td>3b</td>
<td>31M</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>9</td>
<td>NAÏVE</td>
<td>SOF+DAC 24</td>
<td>3b</td>
<td>31M</td>
<td>R</td>
<td>S</td>
</tr>
</tbody>
</table>

**Colour Legend**
- Resistant
- Sensitive
As seen in table 2 above, eight out of nine patients had genotype 3b, while one patient had genotype 3a. All 9 HCV variants extracted from patient samples had NS5A gene sequences resistant to Daclatasvir. The only patient with genotype 3a had a dual NS5A resistance to both Daclatasvir as well as Velpatasvir, which is a part of the second line treatment regime according to the MSF Hepatitis C project (Karachi) clinical protocol. This protocol is updated every year based on WHO guidelines and contextual evolution.

All patients with genotype 3b were treatment-naïve and have a consistent mutation of 31M (which was resistant to Daclatasvir). To re-treat these patients, Sofosbuvir-Velpatasvir-Voxilprevir combination would be required.

The patient with genotype 3a failed treatment on three HCV regimens, once with Peg-interferon + Ribavirin for 6 months, once with Sofosbuvir + Ribavirin for 6 months, and finally again with Sofosbuvir + Daclatasvir 24 week treatment (at MSF Clinic). As this patient is resistant to both Daclatasvir as well as Velpatasvir, a combination of Glecaprevir and Pibrentasvir would be required for re-treatment.

Evidence shows that with multiple treatment exposures, HCV Resistance associated substitution could be either because of de novo substitutions arising and replicated with selective pressure during treatment regimens, or a pre-mutated variant acquired from the community. A baseline gene-sequencing testing would have been required for these 9 patients to establish either of the above.
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Glossary of abbreviations

APRI - Aspartate Aminotransferase to Platelet Ratio Index

CHC - Chronic Hepatitis C

CTP - Child Turcot Pugh

DAA - Direct Acting Antiviral

Dac - Daclatasvir

HCV - Hepatitis C Virus

HIV - Human Immunodeficiency Virus

HUG - Hôpitaux Universitaires de Genève

MSF - Médecins Sans Frontières (Doctors without Borders)

Peg-inf - Pegylated interferon

R - Resistant

RAS - Resistance Associated Substitutions

Rib - Ribavirin

S - Sensitive

Sof - Sofosbuvir

SVR12 - Sustained Virological Response at 12 weeks after treatment completion

Tx - treatment

Vel – Velpatasvir
References

3 Gower E, Estes C, Blach S, Razavi-Shearer K, Razavi H. Global Epidemiology and genotype distribution of the Hepatitis C virus infection. J Hepatol 2014; 61
8 Source Hepamud V.3.1