Coronavirus

Before I start my presentation I would like to give you my background.

I wanted to put in context the discoveries and developments in Virology which occurred during my career and highlight how long it takes from the discovery of a new virus and the development of diagnostic tests. The fact that tests to detect this new virus were developed so quickly is impressive - the expectations of the press and public that a test to determine immunity to the virus should be available quickly is unrealistic.

Format of Presentation

- Virus structure
- Virus replication
- Virus classification
- The emergence of new viruses
- The Host Response
- Diagnostic Testing

Virus Structure

A virus is a small and simple infectious agent It can multiply only in living cells of animals, plants, or bacteria. They range in size from about 20 to 400 nanometres in diameter (1 nanometre = 10-9 meters). By contrast, the smallest bacteria are about 400 nanometres in size.

A virus consists of a single- or double-stranded nucleic acid and at least one protein surrounded by a protein shell, called a capsid. The protein capsid provides protection for the nucleic acid and may contain enzymes that enable the virus to enter its appropriate host cell.

Some viruses also have an outer envelope composed of fatty materials (lipids) and proteins - e.g. coronaviruses. Non-enveloped viruses can be more resistant to changes in temperature, pH, and some disinfectants than enveloped viruses.

Some Definitions:

virion: single virus particle
capsid: the outer protein shell of a virus
capsomere: the subunit of the capsid
peplomers: a glycoprotein spike, on a viral capsid or viral envelope, which bind to specific receptors on the host cell.
envelope: an enclosing structure or cover, such as a lipid membrane
genome: The complete genetic information (either DNA or, in some viruses, RNA) of an organism, typically expressed in the number of base pairs

The corona-like appearance of coronaviruses is caused by so-called spike glycoproteins, or peplomers, which are necessary for the viruses to enter host cells.

The nucleic acid carries the virus's genome —its collection of genes—and may consist of either deoxyribonucleic acid (DNA) or ribonucleic acid (RNA). The vast majority of viruses have RNA genomes. Viral genomes tend to be small, containing only those genes that encode proteins that the virus cannot obtain from the host cell. This genetic material may be single- or double-stranded. It may also be linear or circular.

RNA viruses contain only RNA as their genetic material. To replicate their genomes in the host cell, the RNA viruses encode enzymes that can replicate RNA into DNA, which cannot be done by the host cell. These RNA polymerase enzymes are more likely to make copying errors than DNA polymerases and, therefore, often make mistakes during transcription. For this reason, mutations in RNA viruses occur more frequently than in DNA viruses. This causes them to change and adapt more rapidly to their host. Coronaviruses replicate their RNA genomes using enzymes called RNA-dependent RNA polymerases, which are prone to errors, but genomic analysis so far suggests that covid-19 is mutating slowly, reducing the chance of it changing to become more deadly.

Virus Replication

Viruses use the machinery and metabolism of a host cell to produce multiple copies of themselves.

- **Attachment to the cell**

  Many viruses use some sort of glycoprotein to attach to their host cells via molecules on the cell called viral receptors. For these viruses, attachment is a requirement for later penetration of the cell membrane, allowing them to complete their replication inside the cell. The receptors that viruses use are molecules that are normally found on cell surfaces and have their own physiological functions. Viruses have simply evolved to make use of these molecules for their own replication. Viruses cannot infect cells which do not have these receptors.

  Attachment to the receptor changes the viral envelope protein or capsid protein which result in the fusion of viral and cellular membranes that allow the virus to enter. In the case of coronaviruses the SARS-CoV-2 spike (S) glycoprotein binds to the ACE2 receptor to enter human cells. The full name of the ACE2 receptor is angiotensin-converting enzyme and its purpose is involved with circulation.

- **Entry into the cell**

  Entry of the virus into the cell follows attachment by fusing with the cell membrane

- **Replication of genome**

  The genome replication of most DNA viruses takes place in the cell's nucleus as this is where the host DNA is found. Replication of RNA viruses usually takes place in the cytoplasm. All RNA viruses use their own RNA replicase enzymes to create copies of their genomes. The ssRNA of coronaviruses is similar to mRNA and thus

can be immediately translated by the host cell. Purified RNA of coronaviruses can directly cause infection though it may be less infectious than the whole virus particle.

- **Virus Assembly**

  Current information suggests that the viruses are assembled at the endoplasmic reticulum - a continuous membrane system that forms a series of flattened sacs within the cytoplasm of eukaryotic cells being important particularly in the synthesis, folding, modification, and transport of proteins .

- **Cell death and release of virus particles**

Virus Classification:

Virus classification is the process of naming viruses and placing them into a taxonomic system. Because they are non living they do not fit into the established biological classification system used for living organisms.

- **taxonomy**: the academic discipline of defining groups of biological organisms on the basis of shared characteristics and giving names to those groups.

The International Committee on Taxonomy of Viruses (ICTV) is a committee which authorizes and organizes the taxonomic classification of viruses. They have developed a universal taxonomic scheme for viruses and aim to describe all the viruses of living organisms. Members of the committee are considered to be world experts on viruses.

- The general taxonomic structure is as follows: Order (-virales); Family (-viridae); Subfamily (-virinae); Genus (-virus); Species (-virus).

Members of The Coronaviridae Study Group (CSG) of the International Committee on Taxonomy of Viruses classified the virus tentatively named 2019-nCoV as SARS-CoV-2 by studying its genome.

Family: Coronaviridae

Species: Severe acute respiratory syndrome-related coronavirus

Why are they called coronaviruses?

As the journal *Nature* reported in 1968, "these viruses are members of a previously unrecognized group which [the virologists] suggest should be called the coronaviruses, to recall the characteristic appearance by which these viruses are identified in the electron microscope." The appearance of the virus reminded them of the corona of the sun during a solar eclipse.
Coronaviruses are single-stranded RNA viruses, about 120 nanometers in diameter. They are susceptible to mutation and recombination and are therefore highly diverse. There are about 40 different varieties and they mainly infect human and non-human mammals and birds. Coronaviruses are zoonotic, meaning that they can be transmitted to people from animals. Both SARS-CoV and MERS-CoV originally came from bats, though other animals –

including camels in the case of MERS – can act as intermediaries that spread coronaviruses to humans. Coronaviruses are a group of viruses that usually cause mild illnesses, such as the common cold. However, certain types of coronavirus can infect the lower airway, causing serious illnesses like pneumonia or bronchitis. Most people get infected with coronaviruses at some point in their lives and the majority of these infections are harmless. The new coronavirus that causes the covid-19 illness is a notable exception.

Other severe coronaviruses

At least two other types of human coronavirus – Middle East respiratory syndrome coronavirus (MERS-CoV) and severe acute respiratory syndrome coronavirus (SARS-CoV) – are known to cause severe symptoms.

SARS-CoV first emerged in 2002 in Guangdong, China as an unusual pneumonia, which developed into life-threatening respiratory failure in certain cases. The virus rapidly spread across 29 countries, infecting more than 8000 people and killing about 800.

The MERS-CoV epidemic appeared in Saudi Arabia in 2012, with people experiencing similar symptoms to SARS-CoV but dying at a much higher rate of 34 per cent. Unlike SARS-CoV, which spread quickly and widely, MERS-CoV has been mainly limited to the Middle East.

The Emergence of New Viruses

Why are new human pathogenic viruses continually emerging?

Many of these newly emerging viruses are zoonotic viruses.

In most cases, outbreaks have been known to occur in tropical regions in which there were no human inhabitants. An increase in contact with wild animals, due to the expansion of the human habitat, is believed to be the main cause for the emergence of new viruses. Changes in the environment such as rainforest developments have led to an increase in contact between wild animals and humans. As a result, viruses which only existed in rainforests are able to be transmitted to a new human host.

Climate changes such as global warming are another cause for the emergence of new viruses. In particular, bats serve as reservoirs to many newly emerging viruses. Bats are natural reservoirs for the Ebola virus, SARS-CoV, and the Nipah virus. What makes bats so special? Bats are unique because they are mammals that can fly. As mammals, they are a closer relation to humans than to birds. In addition, it is speculated that the immunity of bats is conspicuously tolerable to viruses. Many types of viruses found in bats are currently being analyzed to provide information about newly emerging viruses that have yet been discovered.

The Host Response

The symptoms of viral diseases result from:

- immune response to the virus, which attempts to control and eliminate the virus from the body
- cell damage caused by the virus

The host defense mechanisms involved in a particular viral infection will vary depending on the virus, dose, and portal of entry. The host has many barriers against infection that are inherent in the organism. These represent the first line of defense. eg. skin, mucus, ciliated epithelium, low pH and the immune response.

What happens when the virus enters the body?

When the virus enters your body it binds to two cells in the lungs - goblet cells that produce mucus and cilia cells which have hairs on them and normally prevent your lungs filling up with debris and fluid such as virus and bacteria and particles of dust and pollen.

The virus attacks these cells and starts to kill them - so your lungs begin to fill with fluid making it hard for you to breathe. This phase of the disease is thought to last about a week.

At this point your immune system will start to kick in and fight off the invaders. You will develop a fever and your high body temperature will create a hostile environment for the virus. You will start to get rid of the mucus in the form of coughing and a runny nose.

But in some people - particularly the elderly and those with other health conditions - the immune system can go into overdrive. As well as killing the virus it also starts to kill healthy cells.

This heightened immune response can trigger a "cytokine storm" - white blood cells activate a variety of chemicals that can leak into the lungs, which along with the attack on the cells damages them even further. Scans of the lungs show "ground-glass" opacity and then "crazy paving" patterns, as they fill with mucus making it harder and harder to breathe.

Bacterial infections can also take hold at this point and your weakened immune system will struggle to fight them off.

This heightened immune response can lead to organ failure and death. It was a common cause of mortality in the Spanish Flu of 1918.

Some people who recovered from severe acute respiratory syndrome (Sars) which swept the world in 2002 to 2003 had long-term respiratory problems as their lungs were permanently damaged. Covid-19 is similar to Sars in some respects, although is much less lethal, so those who have recovered from more serious symptoms may also suffer some long-term effects.

While people with weakened immune systems and the elderly are more likely to become critically ill the younger and healthy in China and elsewhere have also succumbed to the virus - this is because none of us have any immunity to this new disease.

However, one interesting factor is that children do not seem to be falling victim to Covid-19 - just 2.4 per cent of all those who have contracted the disease are 18 and under and the vast majority have mild symptoms. In other respiratory diseases such as flu children are key disease transmitters.

It is still unclear whether children are less susceptible to the disease or whether they just have very mild or asymptomatic infections. According to the World Health Organization there has been no confirmed case of an adult picking up Covid-19 from a child.

Diagnostic Testing

If you have been listening to the daily Coronavirus updates and folloing the BBC website you will feel that you know a lot already about testing for viruses.

As you are probably aware there are 2 purposes for testing - diagnosis and determination of immune status.

To ensure the quality of diagnostic tests the specificity and sensitivity of tests must be determined and positive and negative controls must be included with each batch of tests.

$$\text{Sensitivity} = \frac{\text{True Positives}}{\text{True Positives} + \text{False Negatives}}$$

$$\text{Specificity} = \frac{\text{True Negatives}}{\text{True Negatives} + \text{False Positives}}$$

Diagnosis:

To diagnose viral infections we can detect the virus itself (antigen); its nucleic acid or antibodies produced against the virus. Currently the tests used to confirm a diagnosis of coronavirus is nucleic acid detection. This uses a method called the Polymerase Chain Reaction (PCR).

Often heralded as one of the most important scientific advances in molecular biology, PCR revolutionized the study of DNA to such an extent that its creator, Kary B. Mullis, was awarded the Nobel Prize for Chemistry in 1993.

Sometimes called "molecular photocopying," the polymerase chain reaction (PCR) is a fast and inexpensive technique used to "amplify" - copy - small segments of DNA. Because significant amounts of a sample of DNA are necessary for molecular and genetic analyses, studies of isolated pieces of DNA are nearly impossible without PCR amplification.

Once amplified, the DNA produced by PCR can be used in many different laboratory procedures. For example, most mapping techniques in the Human Genome Project (HGP) relied on PCR.

To amplify a segment of DNA using PCR, the sample is first heated so the DNA denatures, or separates into two pieces of single-stranded DNA. Next, an enzyme called "Taq polymerase" synthesizes - builds - two new strands of DNA, using the original strands as templates. This process results in the duplication of the original DNA, with each of the new molecules containing one old and one new strand of DNA. Then each of these strands can be used to create two new copies, and so on, and so on. The cycle of denaturing and synthesizing new DNA is repeated as many as 30 or 40 times, leading to more than one

billion exact copies of the original DNA segment.

The entire cycling process of PCR is automated and can be completed in just a few hours. It is directed by a machine called a thermocycler, which is programmed to alter the temperature of the reaction every few minutes to allow DNA denaturing and synthesis.

If you remember SARS-CoV-2 is and RNA virus. Before amplification of DNA the viral RNA is converted to its complementary DNA using an enzyme called reverse transcriptase.

Global supply challenges - huge demand for PCR primers and positive controls to ensure the performance of individual machines. The technology to develop a PCR test is relatively simple once the virus sequence is known so a test can be available quickly. The tests can be improved as more sequence information becomes available.
I mentioned earlier that viral infections can be diagnosed by the detection of antibodies produced against a virus. When a virus infects an individual, part of the immune response if the production of specific antibodies. Antibodies are classified into different groups depending on their structure and function. The first group of antibodies produced are IgM antibodies - if they are detected then this indicates that the infection is recent. They can be detected between 7 and 10 days after infection and, in the case of SARS2 lastsfor 12 weeks. Automation for these tests is already available - would be a good method once the test has been evaluated - cheaper than PCR.

Another method is antigen detection - these are also cheaper and easy to automate once the tests have been evaluated. - eg agglutination tests etc

Determination of Immune Status

To reduce the impact of lockdown on individuals and society we need to determine if people are immune to SARS-CoV-2. People with immunity could return to work and borders could be reopened without risk. Medical staff could work without concern for infection and they could return to work if self isolating.

This is done by detecting specific IgG class antibodies. This class of antibodies is produced early in the infection and last many years. The question that scientists still need to answer is how does the viral coat trigger a heathly immune system's recognition and neutralisation of the virus? IgG antibodies to SARS and MERS appear to be long lasting so it is hoped that this will be the case for SARS cOV 2.

Antibody testing:

- verify that vaccines are working as intended during clinical trials
- used as contact tracing weeks or longer after a suspected infection
- help inform public policy makers how many asymptomatic cases have occurred in a population

Unlike PCR tests antibody tests are more difficult to develop.

- Scientists need to know which proteins form the viral coat, specifically those to which the immune system responds, triggering the production of antibodies that flag or neutralise the virus i.e. the antigen
- These sections of the viral coat must then be produced in the laboratory for inclusion in an immunoassay (test which detects antibodies)

Problems:

- Ensuring the protein structure is correct (laboratory produced proteins maybe slightly deformed)

Determining which viral protein to use

- The spike protein is the sole protein on the viral surface responsible for entry into the host cell so it is the main antigen that elicits neutralising antibodies. It is very specific so using this protein reduces false positive results from detecting other coronaviruses e.g SARS
- Scientists from the Institute of Virology at Wuhan are using the nucleocapsid protein and the spike protein. The nucleocapsid protein is the most abundant so is easy to detect

Virologists agree that media reports of re-infection are most likely due to erroneous PCR tests.
The US FDA have already chosen to relax normal assessment criteria.