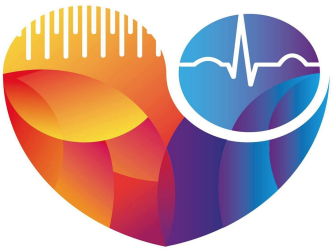
SensUs



GLASGO

Team Results Document

GlasGo

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Summary for the SensUs website

The biosensor developed by GlasGo uses surface acoustic waves to detect and quantify the amount of NT-proBNP in blood plasma. The central piece of the biosensor is a pair of gold interdigital transducers and electrical connectors printed on a piezoelectric substrate. The input interdigitated transducer turns electrical signal into an acoustic wave which propagates to the output interdigitated transducer, across a space called the *delay line*. The acoustic wave behaves like weighing scales: weight added on the delay line modifies the shape (phase or frequency) of the wave. To enable the sensors to detect proteins in blood, such as NT-proBNP, the group added an extra weight in the form of magnetic microbeads. The beads are previously coated with an antibody that binds NT-proBNP molecules passing across them. Using an electromagnet, beads are brought to the sensing surface and then those that are unbound (i.e. do not carry the molecule of interest) are washed away. The change in waveform gives information on how many beads are detected, and this is proportional to the concentration of NT-proBNP in the plasma. The results help diagnose and manage acute and chronic heart failure, as well as predict general cardiovascular risk.

1. Biosensor System and Assay

The GlasGo NT-proBNP biosensor makes use of both biological and mechanical assays. A modified sandwich ELISA is used to transfer mass of microparticles to modify the resonant frequency and phase of a Surface Acoustic Wave (SAW) sensor in relation to the concentration of NT-proBNP in a sample. The modification made to the standard sandwich ELISA lies in replacing the reporter antibodies (fluorophores or HRP) that an enzyme is conjugated to with magnetic microparticles.

A sample containing NT-proBNP is introduced in the well loaded with magnetic microparticles conjugated to the secondary antibody, anti-NT-proBNP. The mix of plasma and magnetic microbeads is pumped along a microchannel, with meander patterns and three electromagnets aiding homogenisation of the solution. The secondary antibodies bind the free NT-proBNP, washing it out of the sample. The solution is simultaneously delivered to two SAW sensors placed side by side: a positive and a control. An electromagnet is then turned on to draw the microparticles to the surface coated with the primary antibodies, where the primary antibody binds the NT-proBNP already attached to the secondary antibody (vice versa may occur). This non-covalent sandwich linkage of the primary antibody from the surface of the SAW device to the magnetic microparticle completes both the ELISA and mass transference. The final step of the assay is a wash phase, similar to the one used in ELISA assays, to remove any unbound microbeads or other molecules from the SAW surface. To ensure accuracy of the measurement, the wash is repeated. Eventually, only the microbeads sandwich-linked via NT-proBNP remain on the surface of the SAW sensor. Experiments can be performed to assess the binding capacity of each bead, hence the total mass of microbeads remaining on the SAW surface is proportional to the concentration of NT-proBNP in the sample. Otherwise, a calibration curve of frequency or phase change can be made using known quantities of NT-proBNP.

SAW biosensors use a piezoelectric substrate (crystal) that is actuated by an electric signal (input), via an interdigital transducer (IDT), and creates a wave that propagates through the surface [1]. This wave interacts with any material present in the detection area and the result is a shift to the wave's frequency, amplitude or phase. Finally, a second interdigital transducer (IDT) is actuated by the wave and creates an electric signal (output) that can be measured and compared to the input. This shift between the input and the output is the means of quantifying any changes in the detection area caused by the presence of the analyte. Certain types of surface acoustic waves, such as Rayleigh waves, are not useful for biosensing because they are highly attenuated when the piezoelectric substrate is in contact with a liquid. More recently discovered Love waves are therefore utilised through the use of a guiding layer of material above the usual substrate layer. This makes the devices sensitive to changes related in mass, viscosity and conductivity in the sensing area above the waveguide.

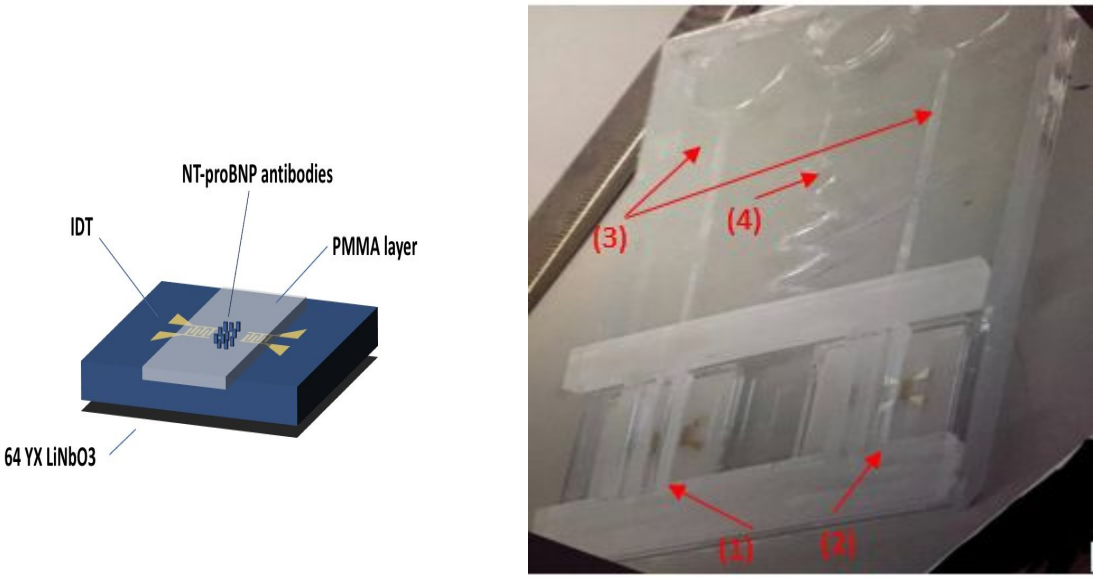

Figure 1: Left - an impression of our device where antibody-conjugated beads will bind NT-proBNP and therefore the functionalised PMMA layer across the IDT; Right - our prototype biosensor with the control and functionalised devices inserted (1 and 2), two wash channels running through the middle of each device (3), and a sample injection point (4). Changes of phase and frequency are then measured remotely.

2. Analytical Performance

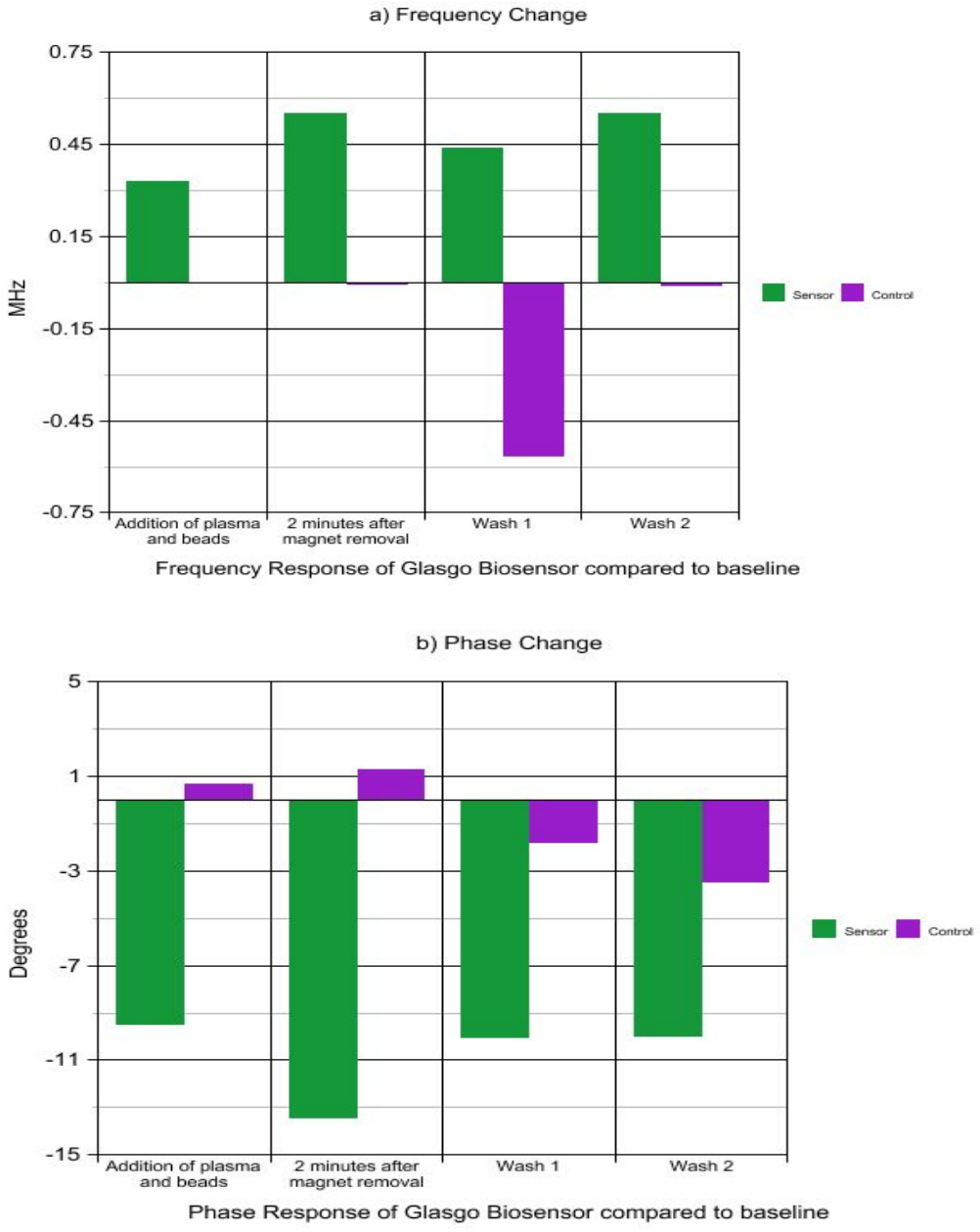

Figure 2: Initial measurements were taken of sensor (antibody-conjugated) and control (non-functionalised) devices, before 20µL of plasma containing 1000pg/ml of NT-proBNP and 10µL of 1ng/ml magnetic beads were added to the biosensor. After addition, magnets drew the magnetic beads to the sensing surface and measurements were taken immediately after these were removed and once more after a further two minutes. Two washes with phosphate buffered saline with 0.1% Tween were then performed, and measurements were taken after each wash. Note: noise after wash 1 for the control device may have made the measurements at this stage slightly inaccurate.

Due to unforeseen circumstances involving device functionalisation and cartridge leakage, the process of calibration to various NT-proBNP measurements was still ongoing at the time of the report production. Figure 1 however illustrates the changes in frequency and phase in the sensing device compared to the control device when 1000pg/ml of NT-proBNP is added. We expect the device to accurately be able to detect NT-proBNP levels above and below the threshold value of 300pg/ml used in acute heart failure diagnosis. Annexe, Figure 3 illustrates the binding of beads and NT-proBNP to the functionalised surface in experiments before manufacturing of our cartridge was completed.

3. Novelty and Creativity

3.1. Already available

GlasGo team used James Watt Nanofabrication Centre facilities as well as photolitography, 3D printing and laser cutting to manufacture key components of the biosensor. The microbeads used were ordered from ThermoFisher. Similar devices on the market include a SAW biosensor marketed by SAW Instruments GmbH [2] and industrial sensors that employ SAW sensing, manufactured by Senseor [3].

3.2. New developments

During the development stage of the project, we evaluated the fundamentals of the approaches that could be employed as the principal sensing technology. The composition of our team made compelling arguments for both a mechanical and a magnetic approach. The mechanical approach was the use of surface acoustic waves because they are sensitive to the picogram unit, cheap to produce, easy to mass produce, miniaturize and test. Meanwhile the magnetic approach promised fast results [4], and had featured in many emerging biotechnology applications, including biosensors [5].

Our biosensor makes use of the strengths of both concepts. Surface acoustic waves are used in the principal assay, to quantify the NT-proBNP in a sample. However, the inclusion of magnetic microparticles introduces greater mass transference, making the change in resonance frequency/phase easier to detect by the acoustic waves; moreover, the use of magnetism allows faster completion of the modified sandwich ELISA and easier washing (see Biosensor System and Assay).

Our modification of the ELISA makes our detection system unique. It was noted that SAWs are not commonly utilised in biotechnology, and while this may deter others, we took this as an opportunity to find the weak spots of this sensing system.

4. Translation Potential

4.1. Healthcare application potential

The biosensor we have designed is intended to be used primarily to aid the diagnosis and management of heart failure, which has a prevalence of more than 1% in the general population [6], both in the acute and chronic setting. Since NT-proBNP measurement has an extremely high sensitivity - but relatively low specificity - for diagnosing acute heart failure [7], we envisage that our biosensor could be used as a 'rule-out' screening tool for doctors working to triage patients coming through the doors in accident and emergency with symptoms such as dyspnoea. Current hospital practice relies on chest X rays and electrocardiograms to aid the diagnosis [7]. Importantly, approximately 50% of chest X rays are normal in heart failure [8], and electrocardiogram changes are nonspecific [9]. Therefore, our biosensor acts as an extra tool to decide whether patients necessitate treatment or further investigation by more expensive, and less readily available, echocardiography. Many patients with developing heart failure also present to their general practitioner – it is estimated that the average general practitioner will suspect new onset heart failure in 10 patients per year [10]. Cost analysis has already shown that natriuretic peptide measurement to decide who should be referred for echocardiography is likely superior to the current practice of referring everyone who is suspected of having the condition [11]. Enabling GPs to check these levels on the spot would therefore not only be cheaper for the NHS, and other national health bodies abroad, but also ensure speedier diagnosis of heart failure by reducing waiting times for echocardiography.

The group designed and gained ethical approval for a survey to deliver to the general public and healthcare professionals about the cost and utility of our biosensor. From cardiologist survey feedback and our own research (Annexe, Figure 4), we have learned that there is currently a poor evidence base for repeated natriuretic peptide measurement in management of chronic heart failure. Some studies have suggested that reduced mortality could be possible with aggressive pharmacological management towards a natriuretic peptide concentration target [12], however the evidence base is still not convincing enough for this management to become common practice. Due to using traditional lab assays, many of these studies only measured natriuretic peptides once every week or month. Giving general practitioners and patients themselves the potential to measure their NT-proBNP levels on a daily basis, tracking and sending data to their consultant via smartphone, could be trialed with our biosensor. If this showed positive outcomes, our biosensor could then be used population-wide to improve the management of chronic heart failure.

On top of its use in heart failure diagnosis and management, we also have a slightly bolder vision of how our biosensor could be used in the future. Current practice in the UK in managing the risk of cardiovascular disease, and the subsequent prescription of appropriate medicines such as cholesterol-lowering statins, relies upon aggregated risk scores [13]. These scores, for example QRISK2, take into account factors such as smoking status, blood pressure, and LDL and HDL cholesterol levels to calculate the likelihood of an individual suffering from a cardiovascular event, such as a heart attack or stroke, in the coming years. Recent evidence shows that natriuretic peptide levels in the general population may actually be a stronger predictor of a future cardiovascular events than LDL cholesterol levels [14]. The speed and convenience of general practitioners to be able to measure NT-proBNP levels using our biosensor means that in the future the concentration of these peptides could be incorporated into these cardiovascular risk scores, with risk reduction medication being prescribed more appropriately as a result. Our biosensor could mean that NT-proBNP measurement in the healthy population could become as quick, easy, and common as blood pressure measurement in general practice.

In all of the described uses, an output to app would be available (Annex, Figure 5) to maximise user friendliness, with sections specific to medical professionals and patients. This was in keeping with survey preferences from a variety of age ranges (Annex, Figures 6 and 7).

4.2. Industrialization and commercialization potential

The end product will take a different shape to the prototype, including having the potential for testing various biomarkers, facilitate mass production, and compete with similar products available on the market such as the Samsung LABGEO PT10 and Roche cobas h232. The roadmap to production involves miniaturisation of the device - especially the phase/frequency analyser - and the mass production of materials at lower costs (described below). Interestingly, due to our use of Love waves in the biosensor, we have a unique marketing avenue of 'Love biosensors for failing hearts'.

In terms of casing and cartridge manufacturing, injection moulding techniques would replace laser cutting to lower time expenditure and costs, since the troubleshooting utility of laser cutting would not be needed. The biosensor itself would be more costly to manufacture if the devices were made from lithium niobate, as they are now - around £100 per device in our lab when raw materials and dicing are included. However, using larger wafers made from cheaper materials, such as zinc oxide, would decrease the cost substantially. Moreover, used devices could easily be recycled in a manufacturer-customer exchange process by simply stripping and recoating the devices with the detection layer. Therefore the only major expense for the customer would be buying the biosensor with the initial devices and electronics, which we envisage could be less than £100 if sold widely (for example, if contracts were made with the National Health Service in the UK then 10,000 could be sold to GP practices alone [15]). There may have to be an initial investment on our part of providing additional SAW devices to use, however this would be recovered through the recycling process. This means that GlasGo sensor would be substantially cheaper than competitor NT-proBNP biosensors, without compromise on variables such as speed of detection. Our public survey data (Annex, Figure 8) shows the majority of patients would be willing to pay this to monitor heart failure and some would pay this to monitor their cardiovascular risk if they were otherwise healthy.

The cartridges could be manufactured at a lower cost by conjugating beads with antibody on an industrial scale. The most costly materials in this case would be: Pierce NHS beads at £684 for 5ml (non-industrial); yielding 2500 cartridges (27p per cartridge); Hytest NT-proBNP antibodies at approximately £400 per 1mg of antibody, which would conjugate enough beads for approximately 5000 cartridges (8p per cartridge). Therefore cartridge cost would be slightly over 35p per cartridge, plus the cost of the recycled SAW devices, which we estimate to be up to £2, including PMMA cost and transport. In this case too, members of the public would be willing to pay for both cardiovascular disease risk testing and heart failure monitoring (Annex, Figure 9).

Using information we gathered from talking to industry specialists such as EPIGEM Ltd and SAW Dx, in the next year a more robust prototype which incorporates the changes presented above could be designed.

5. Team and Support

5.1. Contributions of the team members

After the participants for SensUs were selected, six sub-teams were constituted (Magnetic Nanoparticles, SAW device, Manufacturing, Electronics and Signal Processing, Communication, User Interface) and their respective leaders were chosen.

Theodore Tzirides – GlasGo team leader and leader of the *SAW device* group. Theo arranged weekly meetings, gathered briefings from other group leaders and gave advice and directions to the members. He has designed and manufactured the SAW devices and was involved in the testing of the devices. **Ioana Susnoschi** – leader of the *Manufacturing* group. Ioana designed and manufactured the single-use Perspex microfluidic devices that would transport the fluids to the SAW sensors. She performed testing on the final devices and functionalised PMMA-coated SAW devices. She made the UX for the smartphone application. **Samyak Shah** designed the Phase Locked Loop circuit that sends and receives electrical signal from the SAW devices. He has constructed and programmed motor pumps that are used to drive fluid through the microfluidic devices. He designed the user interface of an application for Android smartphones that would be compatible with the biosensor. **Margarita Ivanova** designed the 3D printed slide-in holder for the single-use sensing devices. She manufactured and tested Perspex microfluidic devices. She functionalised SAW devices and performed ELISA assays. **Thomas Parry** – leader of the *Magnetic Nanoparticles* group. He researched and employed a modified sandwich ELISA that uses magnetic beads instead of reporter antibodies. He coated the magnetic beads with anti-NT-proBNP. **Nathaniel Quail** – leader of *Communication* team, he organised GlasGo's participation at the *Science Lates: Innovate* event at the Science Centre. He researched the medical aspects of the biosensor and made the questionnaire that was given out at the event. He was involved in antibody conjugation to beads and the devices, and performed tests on the SAW devices as well as on the final biosensor. **Vicente Ferrer Gallardo** manufactured and spincoated the SAW devices for the competition. He made the electric circuit that was used to demonstrate the principle of GlasGo's biosensor at the *Science Lates* event. **Theodora Torcea** contributed in manufacturing and testing of the Perspex devices. She was also in charge of competitors' analysis and assisted with poster design for the *Science Lates: Innovate* event. **Izabel Alvares** helped with the modified sandwich ELISA and with the biochemistry for secondary binding of NT-proBNP to the magnetic beads. She managed the social media accounts. **Yujie Zhang** helped make the demo electrical circuit for the Science Centre event and finalised the motor pumps. **Wee Ser Tan** made the electromagnets for mixing of the magnetic beads and plasma sample along the channels. **Anna Nelson** assisted with the *Science Lates: Innovate* poster design.

5.2. People who have given support

Dr. Julien Reboud guided throughout the project, helping with the many queries we had throughout. **Dr. Rab Wilson** gave guidance and advice on the manufacturing of the single-use devices. He helped the team find their way around the lab. **Dr. Andrew Glidle** took care of all the admin matters for the GlasGo team. The staff from James Watt Nanofabrication Centre - **Mrs. Linda Pollock**, **Mrs. Susan Ferguson**, **Mrs. Helen McLelland**, **Mr. Robert Harkins** and **Mr. Jon Humphreys** offered training for fabrication of the SAW devices, but also invaluable suggestions for overcoming any issue during fabrication. PhD student **Miss Alice Garrett** helped the Manufacturing team overcome a couple of

problems and offered some materials. **The staff** from the Rankine building workshop provided the team with materials and performed jobs when required. **Mr. Douglas Irons** printed the posters for the Science Centre event as well as for the competition.

6. Final remarks

We would like to thank the organisers of the SensUs competition for their enthusiasm and the help they offered throughout the challenging and exciting process; for giving us an opportunity to develop our scientific knowledge and skills; and also for giving us the chance to make lifelong friends along the way!

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8. Appendix

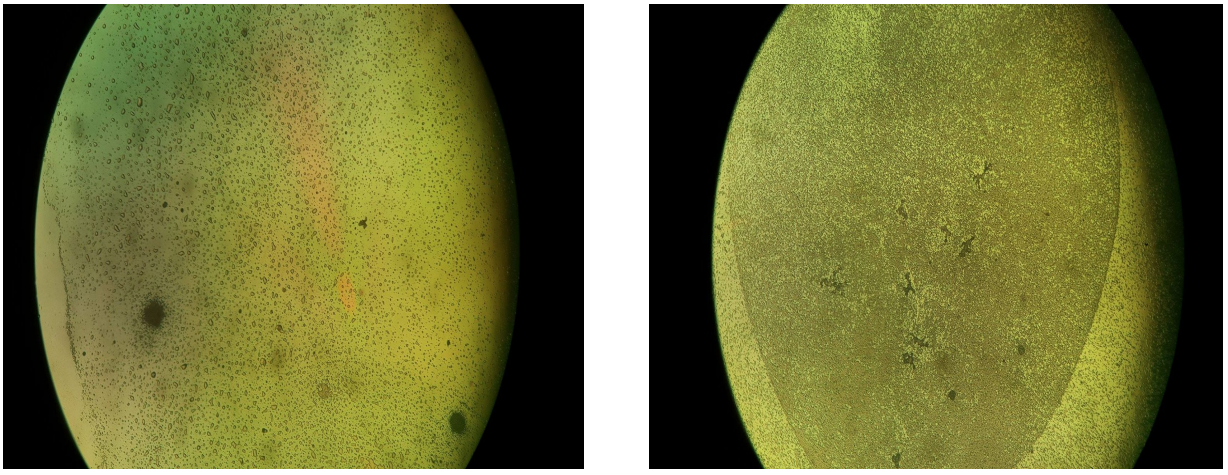


Figure 3: Sandwich assays using antibody-conjugated beads and PMMA surface, and either a) 20µL of 1% BSA, or b) 20µL of 1000pg/ml of NT-proBNP (followed by 3 washes in phosphate buffered saline with Tween). This demonstrates the specificity of the assay for NT-proBNP.

Q3

I think measuring NT-proBNP on a portable device would be useful for... (1 = not useful, 10 = very useful)

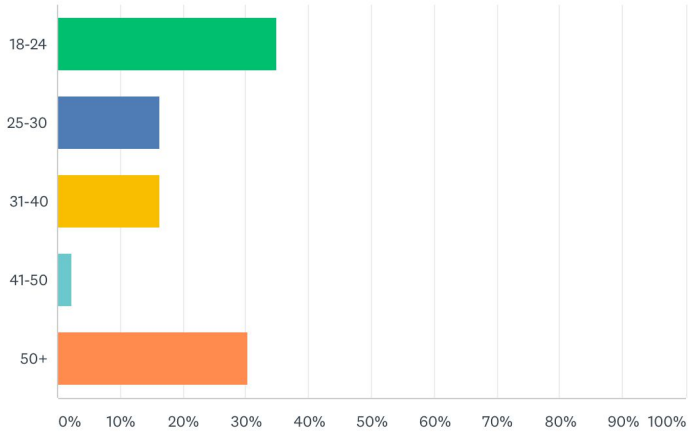
A&E doctors detecting acute heart failure	8
Comment:	Natriuretic peptides (NPs) already available in many EDs however generally lab based. Differentiator here would be testing time reduction.
Paramedics detecting acute heart failure	2
Comment:	Not useful. Pre-hospital blood testing is fraught with risk of misdiagnosis. High NP levels will not change Rx: if pt unwell will go to ED anyway. False negative NP results (which occur) could have disastrous consequences (and anyway, if unwell, pt should still go to ED).
Triage nurses detecting acute heart failure	2
Comment:	In what setting - the ED? If tested by triage nurses would be used even more indiscriminately than is troponin.
GPs detecting heart failure	6
Comment:	V useful for GPs, however why better than doing via a lab assay? Other than faster answer for pt, I am not sure of added value. In pure terms though, NPs will be v valuable tests for GPs if used correctly.
GPs taking regular measurements in chronic heart failure and adjusting medication with cardiology input	2
Comment:	Despite plenty of attempts, there is no convincing evidence yet (that I know of) that NP-guided care improves outcomes. In other words, what is the point of people measuring their NPs at home, or having them monitored by their GP? Some HF specialists do track NPS in clinic, and in a small proportion of patients who are eligible for advanced therapies (basically, cardiac transplant), NPs tracked over time are used to support decision making. However, their evidence based use is currently in the diagnosis (or exclusion) of HF (also useful prognostically), rather than monitoring it over longer term.
Patients taking regular measurements in chronic heart failure for their GP/cardiologist to adjust their medication	2
Comment:	Same as above. Plus asking patients to take their own blood at home sounds problematic.

Figure 4: Example response from cardiologist when asked to rate the utility of a biosensor in various settings.

Figure 5: Template of app developed for Android smartphones, showing sections for doctors and patients, calibration, and statistics (e.g. weight and fluid balance recording by patient). Results can be sent to patient account, allowing specialists to view in real time, regardless of location.

Age range

Answered: 43 Skipped: 0



ANSWER CHOICES	RESPONSES	
18-24	34.88%	15
25-30	16.28%	7
31-40	16.28%	7
41-50	2.33%	1
50+	30.23%	13
TOTAL		43

Figure 6: Age range of respondents to public survey varied widely.

	1	2	3	4	5	6	7	8	9	10	TOTAL	WEIGHTED AVERAGE
Using a smartphone, wirelessly connecting to the sensor	2.38% 1	0.00% 0	0.00% 0	0.00% 0	0.00% 0	0.00% 0	4.76% 2	11.90% 5	9.52% 4	71.43% 30	42	9.31
Using a smartphone, which the sensor plugs into	0.00% 0	0.00% 0	5.13% 2	7.69% 3	2.56% 1	10.26% 4	15.38% 6	23.08% 9	15.38% 6	20.51% 8	39	7.56
A display on sensor itself	0.00% 0	0.00% 0	0.00% 0	10.26% 4	7.69% 3	2.56% 1	15.38% 6	12.82% 5	5.13% 2	46.15% 18	39	8.13
A print out of results	12.82% 5	10.26% 4	7.69% 3	5.13% 2	10.26% 4	12.82% 5	7.69% 3	15.38% 6	2.56% 1	15.38% 6	39	5.59

Figure 7: Public responses generally preferred the option of having results given wirelessly to their smartphone.

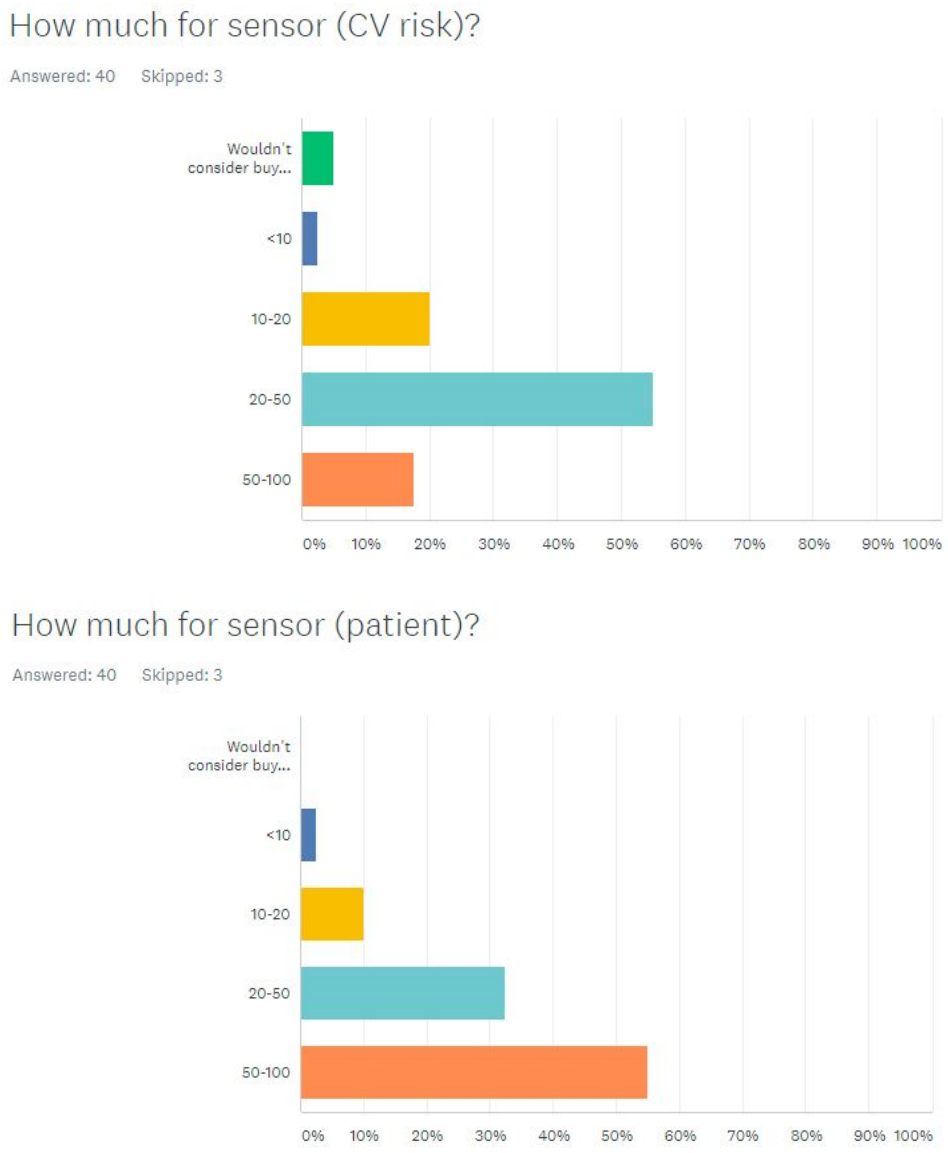
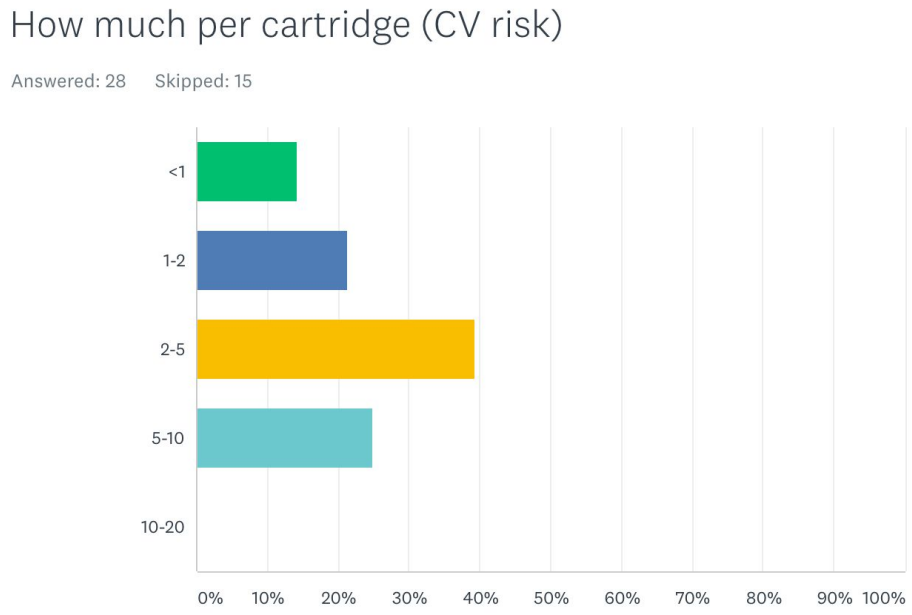


Figure 8: Data from patient surveys showing that the majority would pay approximately £20-50 for a sensor to monitor their cardiovascular risk if they were otherwise healthy (graph 1) , whereas the same population would pay £50-100 for a sensor to manage their treatment if they were diagnosed with heart failure (graph 2).



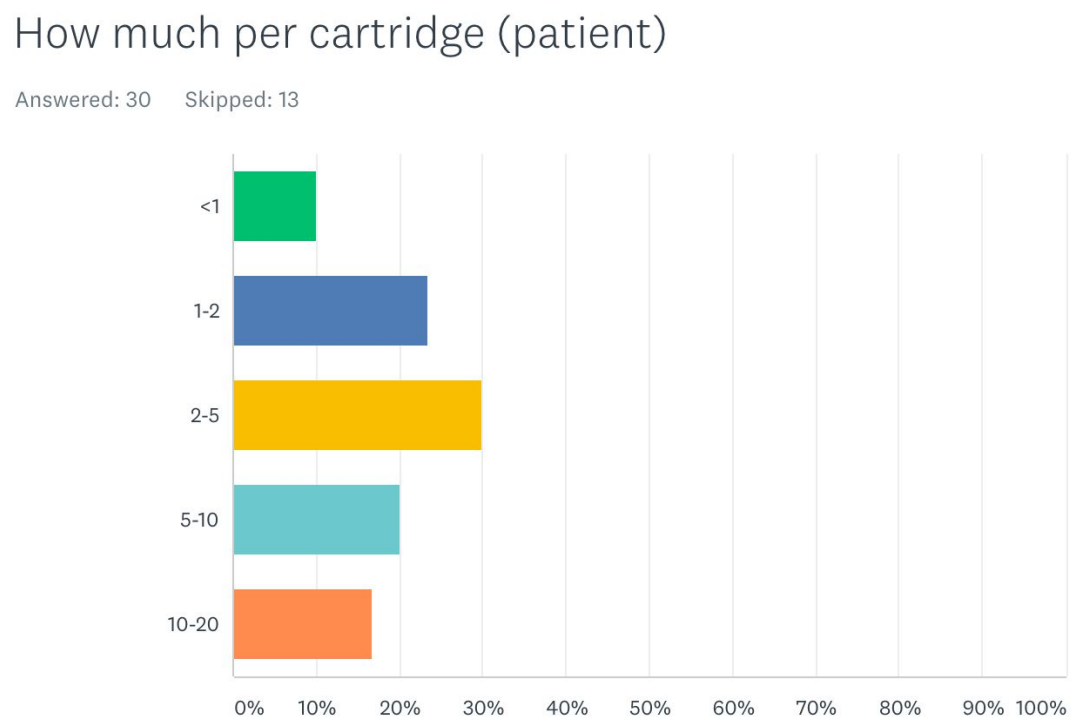


Figure 9: Data from patient surveys showing that the majority would pay approximately £2-£5 for a cartridge to monitor their cardiovascular risk if they were otherwise healthy (graph 1) , with a slight shift towards accepting a higher price per cartridge to manage their treatment if they were diagnosed with heart failure (graph 2).