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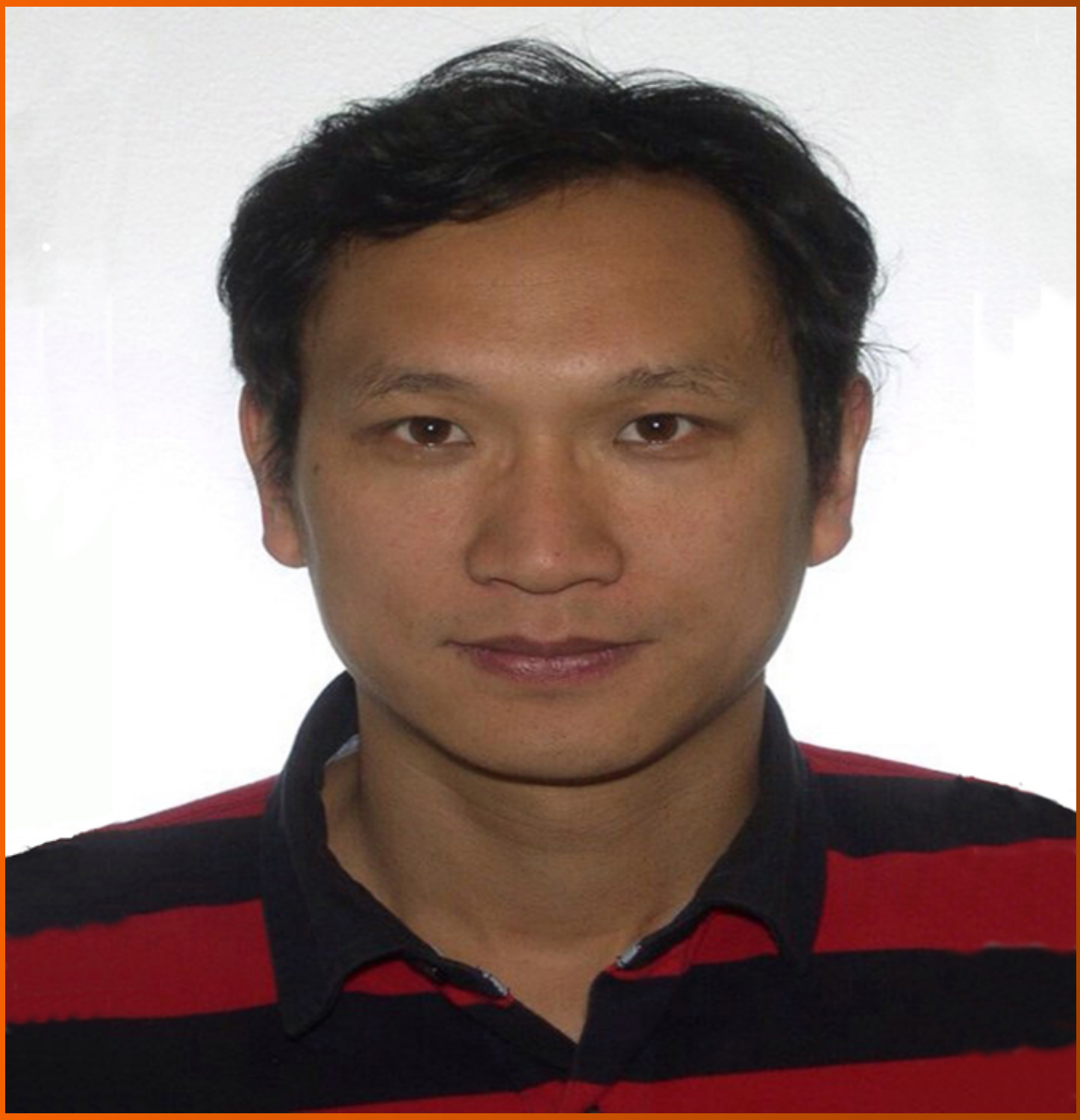

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EDITORIAL

Social media in cardiology: Reasons to learn how to use it

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Abstract

Social media has changed the way we learn, educate, and interact with our peers. The dynamic nature of social media and their immediate availability through our portable devices (smartphones, tablets, smartwatches, *etc.*) is quickly transforming the way we participate in society. The scope of these digital tools is broad as they deal with many different aspects: Teaching and learning, case discussion, congresses coverage, peer to peer interaction, research are examples worth mentioning. The scientific societies considered more innovative, are promoting these tools between their members. These new concepts need to be known by the cardiologists to stay updated, as countless information is moving rapidly through these channels. We summarize the main reasons why learning how to use these tools to be part of the conversation is essential for the cardiologist in training or fully stablished.

Key words: Social media; Cardiology; Congress; Learning; Teaching; Interaction; Cardiovascular diseases; Impact Factor; Portable devices; Smartphone; Tablet

Core tip: Social media has changed the way we learn, educate, and interact with our

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peers. The scientific societies considered more innovative are promoting these tools between their members. These new concepts need to be known by the cardiologists to stay updated, as countless information is moving rapidly through these channels. We summarize the main reasons why learning how to use these tools to be part of the conversation is essential for the cardiologist.

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INTRODUCTION

Social media could be considered as interactive computer-mediated communication tools which have important penetration rates in the general population in middle and high-income countries. Though, in health sciences, many stakeholders (*e.g.*, clinicians, academic institutions, professional colleges, administrators, ministries of health, between others) are unconscious of social media's relevance[1].

Social media has changed the way we learn, educate, and interact with our peers. The dynamic nature of social media and their immediate availability through our portable devices (smartphones, tablets, smartwatches, *etc.*) is quickly transforming the way we participate in society[2].

The scope of these digital tools is broad as they deal with many different aspects: Teaching and learning, case discussion, congresses coverage, peer to peer interaction, research are examples worth mentioning. A good summary was shown by Snipelisky[3] about the 4 main reasons to be involved with social media tools: Personal use, networking, education and public health. There are many others, but these 4 aspects are probably the main points. As other authors have highlighted literacy in the "Digital Age" it is a necessity. The two unquestioned realities of the digital times are that you can produce your online digital story, or someone else will make it for you[4].

REASONS TO LEARN HOW TO USE SOCIAL MEDIA

Social media tools like Twitter could be considered as a new core competency for cardiologists[5]. Why is so important? Twitter can be used to learn, educate, network, and advocate; and these four reasons together give to the social media experts access to great opportunities.

Many authors underline the potential for engagement between peers, there are no boundaries and the communications are near to be immediate[6,7]; even some people believe that mentoring could be possible through social media[8]. Also contact with patients could be done through Social media but we must be cautious with these approach[9].

Another reason to pay attention to these tools is the impact in the cardiology congresses, probably it is the best way to follow minute to minute a congress at home, previously you needed to wait for your partners coming from the Congress or read web chronicles or article publications, now is at the same time all over the world. You only need to search or follow a congress hashtag (like #ACC18 or #ESCCongress or #AHA17), after that you will reach all the content like you were in the congress arena[10,11]. The impact of social media could be measured through the hashtags and is really high as we show in Table 1.

Another motive to be involved in social media is to reach a good knowledge about the current scientific research and discuss it with peers, maybe the discussion in social media could increment the citations of the papers or even to increase the impact of the author in the community when they discuss directly their research[12,13]. In the last years, many journals are adopting a strategy to spread the journal content through social media[14]. New ways to measuring the impact metrics of the research publications through social media are used now, one of the best examples Altmetrics[15], that maybe could compete with the classic impact factor in the future.

Another interesting social media tool that needs to be mentioned is Youtube, as the

Table 1 Impact of Cardiology Social Media by conference hashtag measurement

Conference hashtag	Hashtag registration date[1]	Total tweets[2] (thousands)	Total retweets (thousands)	Total participants (thousands)	Digital impressions[3] (millions)	Visuals[4] (thousands)	Papers[5] (thousands)
#AHA17	06/29/17	62.0	42.4	17.4	339.1	44.5	17.7
#ACC18	12/11/17	51.4	35.6	10.1	372.5	42.2	14.8
#ESC18	12/29/17	54.5	20.0	23.8	137.5	17.9	4.6

[1]Registration date reflects the date the hashtag was registered with symplur.com. Individual hashtag data is from the registration date to access on September 22, 2018;
[2]The total number of unique tweets since the hashtag was registered on symplur.com;
[3]Impressions are computed by taking the number of times an account has tweeted multiplied by the account's number of followers repeated for all accounts, then finally summed up;
[4]The total number of times each photo, GIF, or video was shared;
[5]The total number of papers or links/URLs shared. Data from Symplur signals[23].

second common search engine after Google. Many journals or scientific societies are using it for the dissemination of content and interaction with their potential audience[16].

If you are an academic leader probably you need to embrace the social media tools as there is a need for leadership on the social media discussions; the classic leaders are reluctant to abandon the typical forums of debate and the discussion it will be not there again, the audience is worldwide and the way to discuss is quickly changing[17,18].

For sure the future research will be about social media use, and it will focus on the impact on public health and the education of patients without any doubt. It is not noise it is a great opportunity[19-21].

CONCLUSION

The scientific societies considered more innovative are promoting these tools between their members. These new concepts need to be known by the cardiologists in training or fully stablished to stay updated, as countless information is moving rapidly through these channels. Do as the cardiology leadership is doing and don´t stay away from social media, there are more benefits than threats there[22].

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REVIEW

Cellular models for human cardiomyopathy: What is the best option?

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Abstract

The genetic cardiomyopathies are a group of disorders related by abnormal myocardial structure and function. Although individually rare, these diseases collectively represent a significant health burden since they usually develop early in life and are a major cause of morbidity and mortality amongst affected children. The heterogeneity and rarity of these disorders requires the use of an appropriate model system in order to characterize the mechanism of disease and develop useful therapeutics since standard drug trials are infeasible. A common approach to study human disease involves the use of animal models, especially rodents, but due to important biological and physiological differences, this model system may not recapitulate human disease. An alternative approach for studying the metabolic cardiomyopathies relies on the use of cellular models which have most frequently been immortalized cell lines or patient-derived fibroblasts. However, the recent introduction of induced pluripotent stem cells (iPSCs), which have the ability to differentiate into any cell type in the body, is of great interest and has the potential to revolutionize the study of rare diseases. In this paper we review the advantages and disadvantages of each model system by comparing their utility for the study of mitochondrial cardiomyopathy with a particular focus on the use of iPSCs in cardiovascular biology for the modeling of rare genetic or metabolic diseases.

Key words: Cardiomyopathy; Mitochondria; Induced pluripotent stem cells; Fibroblasts; Cellular models

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Core tip: Several experimental model systems exist for the modeling of cardiomyopathies, including those caused by rare metabolic or mitochondrial diseases. We compare and contrast the cellular models that have been used to date to model several different mitochondrial disorders with a particular focus on the advantages and disadvantages of induced pluripotent stem cells.

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INTRODUCTION

The cardiomyopathies are defined as a group of diseases of the heart characterized by abnormal structure and function of the myocardium[1]. The cardiomyopathies have been classically grouped according to cardiac morphology with the major categories being: hypertrophic cardiomyopathy (HCM), restrictive cardiomyopathy, dilated cardiomyopathy (DCM), arrhythmogenic right ventricular cardiomyopathy and left ventricular non-compaction cardiomyopathy (LVNC)[2]. These groups can be further subdivided into genetic and acquired forms based on disease mechanism[2]. The genetic cardiomyopathies generally arise in childhood or early adulthood and include metabolic and monogenic diseases.

The inborn errors of metabolism (IEM) are a heterogeneous group of rare genetic diseases caused by defects in energy production or intermediary metabolism[3,4]. Within the pediatric cardiomyopathies, IEM affect between 5% and 26% of infants and children[5]. There are more than 40 different IEM that are associated with the development of cardiomyopathy[3]. The mitochondrial cardiomyopathies represent the largest subset and result from pathologic mutations in either mitochondrial or nuclear genes[6] that ultimately lead to dysfunction of the electron transport chain[7], the main supplier of cellular energy under aerobic conditions[8]. Since the heart is one of the most energy-demanding organ in the body[9], cardiomyopathies are found in 20%-40% of children with mitochondrial disease[10]. Given the early onset of these devastating multisystem diseases, research into disease mechanism and the identification of potential therapeutics is essential. However, the heterogeneity and rarity of the IEM and the mitochondrial cardiomyopathies preclude randomized clinical drug trials with standardized end-points. This makes disease modelling using animals or cells an essential component in the study of these diseases.

ANIMAL MODELS

The use of animal models for research, with rodents in particular, continues to represent the most commonly used and successful approach in reductionist biology. However, despite its many successes, this methodology is still questioned because of ethical implications, the frequent inability to totally recapitulate human genetic variability[11] and the fact that important species-specific differences exist for many aspects of biology which complicate both the study of disease and the translation of therapies into human subjects[12]. For example, in cardiac research specifically, the use of rodent models may be limited due to substantial biological differences in the cardiovascular system between rodents and humans. Rodent hearts beat at considerably higher heart rates (200-300 beats per minute) than humans (60-100 beats per minute)[13] and the duration of the ventricular action potential is significantly shorter in rodents[14] compared to humans[15]. Additionally, cardiomyocytes differ in the proteins expressed in the myofilaments, which affects repolarization and calcium sensitivity[13]. One potential strategy to improve the utility of animal models is to create "humanized models" using genetic engineering[11] or engrafting animals with human cells or tissues and immune suppressing them to prevent rejection of the foreign material[16]. Although this type of model is useful for studying many conditions including cancer[17], infectious diseases[18] and liver disease[19], they have important limitations, especially in terms of time, cost and difficulties in creation and maintenance. Furthermore, these hybrid animal models are often not feasible for studying the heart and cardiovascular system.

CELLULAR MODELS FOR CARDIOVASCULAR DISEASE

The adult mammalian heart is composed of multiple cell types, including cardiomyocytes, fibroblasts, endothelial cells, vascular and perivascular cells. The composition of the heart varies greatly between species[20] but, in humans, cardiomyocytes are the dominant cell type by volume, encompassing 70%-85% of the total heart. Cardiomyocytes give rise to specialized cells such as atrial myocytes, ventricular myocytes and Purkinje cells[21] and are responsible for the generation of contractile force[22]. However, although the other cell types only account for a small portion of the overall total myocardial mass, they are essential for maintaining homeostasis by providing the extracellular matrix and intercellular communication networks necessary to ensure proper cardiac function[23]. Although cardiomyocytes may be dominant by volume, they are not the most abundant cells. Fibroblasts are actually the most common cell type in the heart and are vital for maintaining the structure, mechanical and electrical functions of the heart[24]. Cardiomyocytes and fibroblasts are the best-studied cardiac cells and, since both cell types have important functions in the heart, we would suggest that both need to be examined to fully comprehend the cardiomyopathies.

Cell culture, using cardiomyocytes, fibroblasts and other cardiac-related cells, represents another well-established system to study human biology, understand disease and assess response to therapeutics. Primary cells and immortalized cell lines derived from human tissues represent two commonly-used experimental models. Primary cells reflect disease biology most faithfully since they are directly isolated from the tissue of interest and they maintain the morphology, function and protein markers in the dish as they possessed *in vivo*, but they are relatively delicate cells that can be difficult to maintain in culture and have a finite lifespan with limited potential for expansion[25]. Immortalized cells are derived by altering cell-cycle check points or modifying telomerase activity and, although these cells don't have a limited lifespan and are capable of sustained active proliferation, they frequently contain genetic aberrations that can accumulate over time and lead to cellular behaviours that are distinct from those demonstrated *in vivo*[26].

Another approach to model disease involves the use of patient-derived cells. These cells are obtained from an individual patient and therefore allow for the study of human disease in its original genetic context and also have important advantages over primary or immortalized cells. The two most commonly used patient-derived cell types used for research today are induced pluripotent stem cells (iPSCs) and fibroblasts. Given that the genetic background for an individual is preserved, the use of these patient-specific cells represents perhaps the best tool to realize personalized medicine[27]. Personalized medicine refers to a health care approach which recognizes each person's distinct genetic, clinical and environmental history[28]. Personalized medicine ideally adapts therapeutics in order to ensure the best response and safety for the treatment of specific diseases with an individualized approach[29]. Using patient-specific cells can help realize this vision by helping researchers identify and understand individual differences.

In conclusion, there are important differences between model systems (Table 1), with advantages and disadvantages that are often dependent on the condition being studied. In reality, a combination of models enabling both *in vivo* and *in vitro* studies is often required. In this paper, our main focus will be to discuss and compare the different cell types which could be useful for studying genetic cardiomyopathies as an alternative to primary cardiac cells. We will illustrate our discussion with examples of mitochondrial cardiomyopathies that have been studied using different cellular models.

IMMORTALIZED CELL LINES

Immortalized cells are defined as cells whose proliferative capacity has been enhanced using different methods[30]. There are a variety of established approaches to immortalize cell lines including the introduction of oncogenes[31-33], viral transformation[34,35], the inactivation of tumor suppressor genes[36,37] or the inactivation of telomere-controlled senescence[38]. The establishment of immortalized cell lines has helped the scientific community to study different biological and molecular events[26], although, this approach has been questioned since these immortalized cells differ significantly from cells with an intact cell cycle control and they are more similar to malignant cells in many respects. Therefore, the results obtained with these cells can potentially be misleading if these differences are not considered[39]. However, the use of immortalized cells still remains one of the most popular models for the study of

Table 1 Comparison between animal and cell models

Properties	Animal	Cellular
Maintain genetic background	No	Yes
Cost of maintenance	Expensive	Less Expensive
Ease of maintenance	Simple	Difficult
Time required	+++	+
Drug effects	Potentially not translatable	Translatable
Study of paracrine effects	Yes	No
Study of circulatory effects	Yes	No

disease.

Immortalized cells have been used to study two inherited diseases caused by point mutations in mitochondrial DNA (mtDNA), mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes (MELAS), and myoclonic epilepsy and ragged-red fibres (MERRF). In both diseases, an alteration in the post-transcriptional modification of a uridine located in an essential position of specific mitochondrial tRNAs, causes oxidative phosphorylation impairment that leads to the inability to generate sufficient ATP to meet the energy demands of the cell[40]. These mitochondrial disorders can be caused by mutations in several genes but, in this example, the immortalized cells were used to model the effect of an A>G transition at nucleotide 3243 in the $tRNA^{Leu}$ gene causing MELAS[41] and a A>G change in the $tRNA^{Lys}$ gene at position 8344 causing MERRF[42]. Two different studies recapitulated these diseases using cybrid cells[43,44]. Cytoplasmic hybrid cells (cybrid) are created using a recipient cell line called rho-zero cells, whose mtDNA has been depleted but the nuclear DNA remains intact and a donor cell which provides mtDNA to the union[45]. This approach has the advantage of being able to isolate mtDNA from a donor patient with a specific mtDNA mutation, allowing for the study of the pathology in an immortalized cell line.

Another rare human disorder, Barth syndrome (BTHS) was studied using immortalized cell lines. BTHS is an X-linked recessive disorder characterized by early-onset cardiomyopathy (usually LVNC or DCM), skeletal muscle weakness and neutropenia related to abnormal mitochondrial structure[46]. Disease severity is highly variable, with patients ranging from being asymptomatic to having severe cardiomyopathy and end-stage heart failure[47]. Studies have shown that BTHS is caused by loss-of-function mutations in the tafazzin (*TAZ*) gene[48]. *TAZ* is a phospholipid transacylase located in the inner mitochondrial membrane and is responsible for remodeling of the phospholipid cardiolipin[49] which is an essential component of the mitochondrial membrane[50,51]. The *TAZ* gene consists of 11 different exons[52] and mutations have been identified in each exon, primarily missense mutations, although small insertions and deletions have also been found[53].

To study BTHS, the authors used a myoblast cell line (C2C12) derived from mouse skeletal myoblast cells, which is commonly used as a model of disease in mammals for skeletal muscle disorders and myopathies[54,55]. The authors designed a stable TAZ knockout (KO) using clustered regularly interspaced short palindromic repeats (CRISPR) technology to target exon 3 in mouse TAZ and cloned it into a plasmid together with the Cas9 nuclease and co-transfected into the cells with a plasmid that allowed for selection with puromycin[56]. With the introduction of the plasmids into the cell, the guide RNA binds to exogenous exon 3, and this binding is recognized by the nuclease, which performs the cutting of the gene, disrupting it. The clone whose genomic TAZ DNA band was fragmented into three pieces was the chosen one to be the model of the disease. According to the authors, this model served to recapitulate BTHS, being consistent with other previous models, showing mitochondrial defects such as accumulation of monolyso-cardiolipin, impaired mitochondrial respiration and increased mitochondrial ROS species[56].

Although these studies have used different immortalized cell models, these might not be the best tool to recapitulate the diseases with accuracy. First of all, these cells are derived either from tumors or from the immortalization of other cell times where the cell cycle or the telomerase activity is compromised, therefore, these cells do not resemble normal cell lines in terms of replication and lifespan and, consequently, this can cause genetic and phenotypic variation over time leading to create heterogeneity in the same cell line[57]. Secondly, these cell lines, like all cell lines are vulnerable to contamination (*e.g.*, Mycoplasma) which can remain undetected and modify cell behaviour and gene expression[58]. Finally, the use of cellular models generated by

using techniques that knockout a gene in particular in a cell line, might not be sufficient to recapitulate the entire spectrum of disease since additional genetic modifiers are not reproduced.

FIBROBLASTS

Fibroblasts are the major stromal cell-type present in connective tissue and are characterized by a flattened and elongated shape with a central nucleus[59] (Figure 1). They are derived from mesenchymal precursors and are part of a heterogenous collection of cells widely distributed over the body. Fibroblasts play an important role in connective tissue by producing extracellular matrix compounds, principally collagen type I and III. Fibroblasts not only have a structural role but they are able to repair damaged tissue by migrating to the site of injury and rapidly proliferating to restore the wounded area[60]. This proliferation potential explains why fibroblasts are so widely used and why they grow *in vitro* very easily[61]. In addition to their growth-related properties, fibroblasts are also increasingly recognized as an important contributor to cardiac biology through cell-cell signalling and physical interactions[62,63]. Unfortunately, fibroblasts have distinct electrophysiological properties and these cells are not electrically excitable despite the presence of multiple ion channels, including potassium and sodium channels[64]. Fibroblasts also lack a specific cell surface marker that distinguishes them from other cell types[65]. However, they can be isolated from a skin biopsy and grown in culture[66] but they do have a limited lifespan[67], so their use to study function, structure and disease mechanism is limited to cells that have not undergone an excessive (< 20) number or cell divisions or passages[66].

Fibroblasts have also been used to study MELAS and MERRF. This study demonstrated that the tRNA point mutations did not modify the number of normal mitochondria but there were important differences found regarding the number of secondary lysosomes and residual bodies in both diseases compared to the control cells[68]. Furthermore, in both diseases, there was impaired respiratory enzyme activity which decreased mitochondrial respiration rate and membrane potential and impacted cell viability due to the inability to synthesize enough ATP to meet the energy requirements of the cell[68]. Even though the cell types affected by MELAS and MERRF in humans are mainly neurons and myocytes[69,70], the easily obtainable skin fibroblasts were sufficient to provide a helpful model to understand some of the mechanisms by which these cell types are compromised. Fibroblasts were also used in BTHS to help understand the molecular basis of the disease. As previously mentioned, diverse mutations have been found in each exon of TAZ, however, there is no clear correlation between the gene mutation type and the different patient phenotypes[71]. The authors used fibroblasts from pediatric patients to correlate the severity of the disease with cellular lipid abnormalities and found that there was abnormal composition of cardiolipin, phosphatidyl-choline and phosphatidylethanolamine[72]. In this study fibroblasts allowed the distinct lipid composition for each patient to be characterized, which enabled insight into the phenotypic complexity of the disease[72].

Although all these studies successfully used fibroblasts to analyze different mitochondrial cardiomyopathies, all studies had to work within the limitation of fibroblast passage number. The passage number refers to the number of times that the cell can undergo cell division and replication. Studies have shown that, with every passage, the number of mitochondria decreases and that there are changes in the structure of these organelles[73]. If not recognized and controlled for, these changes have the potential to mislead researchers into making false conclusions regarding mitochondrial morphology and function.

IPSCS

iPSCs were first created in 2006 after Shinya Yamanaka successfully reprogrammed adult mouse fibroblasts into iPSCs by introducing the pluripotency factors Oct3/4 (Octamer binding transcription factor 3/4), Sox2 (sex determining region Y)-box 2), c-Myc and Kfl4 (Kruppel Like Factor-4) under embryonic stem cells (ESC) conditions[74]. ESCs are derived from the inner cell mass of mammalian blastocysts and possess self-renewal capacity, the ability to grow with an unlimited lifespan and the ability to maintain pluripotency and differentiate into every cell type of the three germ layers[75,76]. The iPSCs created with these "Yamanaka factors" showed the morphology (Figure 2), proliferative properties and gene expression associated with pluripotency

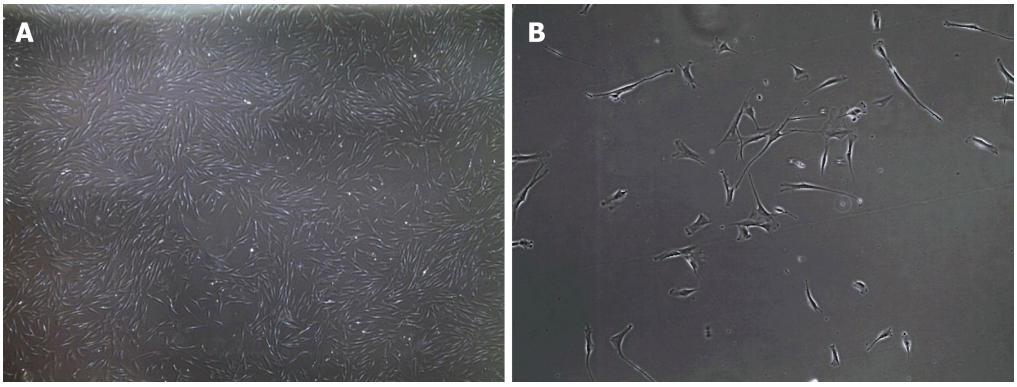

Figure 1 Bright field microscopy images of human fibroblasts. A: 4 × magnification; and B: 20 × magnification.

in ESCs[74] but, importantly, did not have to be derived from discarded human embryos. Currently, iPSCs can be created from a variety of mature, differentiated cells most commonly fibroblasts and peripheral blood mononuclear cells[77].

There are several technical approaches for the delivery of the four critical pluripotency factors necessary for cellular reprogramming to occur[78]. There are integrating methods that include retroviral transduction[74], lentiviral delivery[79] and non-integrative methods such as adenoviral transduction[80], plasmid DNA (episomal) transfer[81], lox p lentivirus delivery[82], Sendai virus delivery[83], piggyBAC transposon[84], protein-mediated (polyarginine-tagged polypeptide)[85] and modified synthetic mRNA[86] (Table 2). Each methodology has its advantages and disadvantages[87-89] and the choice of delivery vector can have important implications in downstream applications and, therefore, needs to be considered carefully.

Once created, iPSCs have significant advantages compared to other cell types as a model of disease. Since they possess the ability to self-renew, there is no concern about how many passages the cells can tolerate and these cells can be relatively easily expanded *in vitro* and be used for many experiments[90]. Furthermore, since they can be differentiated into mostly every cell type[91], researchers can generate patient-disease- and tissue-specific cells for the disease of interest.

DIFFERENTIATION of IPSCS INTO CARDIOMYOCYTES

Most applications using iPSCs to study human heart disease have differentiated them into beating cardiomyocytes[92] although one group (discussed later) took a rather unique approach and differentiated the iPSCs back into fibroblasts[93]. There are several different published and commercial methods to differentiate iPSCs into cardiomyocytes all of which are generally based on the signaling factors that are part of the developmental pathway of cardiomyocytes *in vivo*[94-96] (Figure 3).

Although the ability to generate patient- and disease-specific beating cardiomyocytes is a powerful tool for the study of individual cardiomyopathies[97], the cardiomyocytes that are generated using current methods do have some limitations. First of all, following differentiation, the final population of cardiomyocytes are not completely homogeneous. Differentiated cells contain a mixture of atrial, ventricular and Purkinje cell-types with variable functional properties[98]. If a homogeneous population is desired, it may be necessary to select for the cellular subpopulation of interest using sorting techniques based on surface marker expression[99] or genetic selection[100] which further complicates the process requiring additional time and expense and exposes the cells to additional handling and stresses which they may not survive. Furthermore, for some cell types, *e.g*. ventricular myocytes, unique cell surface markers do not exist[101]. Another issue is that the cardiomyocytes obtained using current differentiation strategies have a phenotype resembling fetal cells in terms of structure, molecular markers and metabolism[102]. This lack of maturity can require additional steps (which are not fully established or reliably reproducible at this time) or additional time in culture to obtain a more adult-like cardiomyocyte population[103] Several methods to stimulate the maturation of iPSC-derived cardiomyocytes have been published based upon electrical[104], mechanical[105], chemical stimulation[106] or matrix modification[107]. This is currently an area of active investigation and future advances and improvements are certain which will further enhance the utility of iPSC-CMs for the study of genetic cardiomyopathies. However, even with these functional limitations of derived cells, they have been helpful for

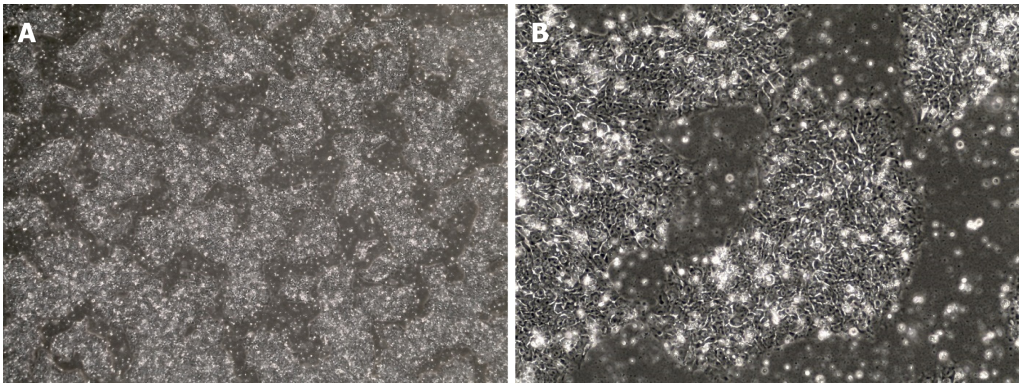

Figure 2 Bright field microscopy images of human induced pluripotent stem cells. Cells display a round morphology with a large nucleus and grow firmly packed in colonies. A: 4 × magnification. B: 20 × magnification.

scientists seeking insight into cardiac biology and disease[108,109].

STUDYING GENETIC CARDIOMYOPATHIES USING IPSCS

Primary fibroblasts from a patient with MELAS were reprogrammed into iPSCs using a retroviral approach in order to establish a novel disease model[110]. As standard practice, the differentiation capacities of the iPSCs were tested using a teratoma formation assay to demonstrate that the cells were capable of generating all germ layers and immunocytochemistry for the pluripotency markers Oct-4 and SSEA-4 was performed to confirm pluripotency. Tissues in MELAS patients can vary in the levels of abnormal mitochondria (heteroplasmy)[111] so the researchers assessed this in patient cells using quantitative real-time PCR to measure mutation ratios and mtDNA copy number. They found that different fibroblast lines had different levels of heteroplasmy ranging from < 5% to 95%. They then demonstrated that those fibroblasts with lower levels of heteroplasmy showed increased heteroplasmy after several passages while those with higher levels did not vary significantly after multiple passages. There were also variations with regards to mtDNA copy number after each passage. This data suggests that the mitochondrial abnormalities in patient fibroblasts can change over time in culture. However, because of their importance in cardiac biology, the authors still wanted to study MELAS. Therefore, the MELAS iPSCs were differentiated back into fibroblasts but, because of the unique self-renewing properties of iPSCs, the authors could overcome passage-associated changes in the mitochondria. In the fibroblasts derived from patient iPSCs, levels of heteroplasmy were found to be similar to the iPSCs from which they were differentiated. These iPSC-derived fibroblasts were then characterized with regards to the enzymatic activities of the mitochondrial respiratory complexes and compared to primary skin fibroblasts. These studies revealed that the iPSC-derived cells recapitulated the disease phenotype and did not demonstrate altered levels of heteroplasmy in culture and therefore represent a unique and novel *in vitro* model of MELAS[110].

MERRF has also been studied using retrovirus-reprogrammed iPSCs. In this study, they generated iPSCs from patient dermal fibroblasts. After reprogramming the fibroblasts using OCT4, SOX2, KLF4, and GLIS1 delivered into the cells, they differentiated the resulting iPSCs into the two different cell types most involved in the disease, cardiomyocytes (iPSC-CMs)[112] and neural progenitor cells (iPSC-NPCs). When they tested all three cell types, they found that all MERRF patient-derived cells (iPSCs, iPSC-CMs and iPSC-NPCs) had reduced oxygen consumption, elevated reactive oxygen species (ROS), reduced growth and fragmented mitochondria. The cellular phenotype correlated with the molecular mechanism of the disease, allowing iPSCs and iPSC-derived cells to serve as a model for the disease[93].

Differentiated iPSCs have also been used in the study of BTHS. The cells of two unrelated patients were reprogrammed using either retroviral[113] or modified RNA approaches[114]. These two patients had different mutations in TAZ, one having a frameshift mutation and the other a missense mutation. After the generation of the iPSCs, they differentiated them into cardiomyocytes that they then used to create tissue layers and a heart-on-chip model[115]. The iPSC-CMs showed abnormalities in cardiolipin processing, sarcomere assembly, myocardial contraction, ROS production and cardiomyocyte functioning, correlating with the abnormalities and cardiac

Table 2 Methods of delivery for reprogramming factors

Method	Advantages	Disadvantages
Retroviral transduction	Efficient, validated for multiple cell types, easy	Transgene integration
Lentiviral delivery	Very efficient	Transgene integration
Adenoviral transduction	Does not integrate	Low efficiency, only validated for fibroblasts
Plasmid DNA transfer (episomal)	Good efficiency, does not integrate, able to replicate autonomously, validated for multiple cell types	Low efficiency in fibroblast reprogramming
Lox p lentivirus delivery	High efficiency, excision of the integrated sequence, gene expression profile closer to hES cells	Genomic instability and genome rearrangements and loxP site remains integrated
Sendai virus	Efficient, does not integrate, validated for multiple cell types	Cost if purchased commercially or challenging if generated by a laboratory
PiggyBAC transposon	Efficient, precise and efficient self-excision, does not remain integrated	Published work only in fibroblasts, licensing patent issues, pBt gene may remain active post-transposition
Polyarginine tagged polypeptide	Does not integrate	Low efficiency, time-consuming, technically challenging and work only on fibroblasts
RNA modified synthetic mRNA	Very efficient, does not integrate, factor available commercially	Cost if purchased commercially or challenging if generated by a laboratory and work only on fibroblasts

dysfunction observed in patients, demonstrating again that is possible to use an *in vitro* model to provide insight into human disease and test potential therapeutics[116].

iPSC-CMs have also been used to study other cardiomyopathies. For example, iPSC-CMs have also been used to understand the pathological effects caused by the reduced expression of frataxin (FXN) in Friedreich ataxia (FA). This neurodegenerative disease is caused by the expansion of a short tandem repeat (GAA) in the *FXN* gene, which can result in transcriptional silencing[117] and therefore, the development of HCM[118] which is an important component of the disease phenotype but its development is not understood. In this study, the researchers generated iPSCs from three patients using an episomal reprogramming approach and then differentiated the resulting iPSCs into cardiomyocytes[119]. Analysis of the iPSC-CMs showed that these cells had an increased beating rate which was related to a defect in calcium handling. Therefore, these cells revealed novel biology that could potentially contribute to the future development of treatment for this disease[120]. It is important to note that this cellular phenotype could arguably not have been accomplished with any other cell type.

The DCM with ataxia syndrome (DCMA) is an autosomal recessive disorder caused by mutation in DNAJC19 and is characterized by 39% mortality[121] during early childhood due to severe heart failure[122]. DCMA has been related to BTHS due to the presence of metabolic abnormalities (*i.e.* production of 3-methylglutaconic acid) and abnormal mitochondria are thought to be responsible for heart failure[123]. Rohani *et al*[123] successfully established four patient iPSC lines that have been differentiated into CMs expressing cardiac-specific markers and this will allow for the study of four unique patient cell lines. This disease still needs to be further characterized but the use of iPSC-CMs derived from patients looks promising as a cellular model to provide a better understanding of the disease.

Finally, iPSC-CMs have also been used to study familial HCM, characterized by thickened left ventricular walls, myofiber disarrays and myocardial fibrosis that often results in arrhythmias[124]. This can be caused by different mutation in at least 11 different genes which encode sarcomeric proteins[125]. In this study, the authors generated iPSC-CMs derived from an HCM patient that had a single missense mutation in the β-myosin heavy chain (*MYH7*) gene. Whole transcriptional analysis of these iPSC-CMs provided useful insights into the disease, revealing important signaling pathways implicated in the pathogenicity of HCM[126].

FIBROBLASTS *VS* IPSCS

As we have described, both fibroblasts and iPSCs have been used to model genetic cardiomyopathies and both cell types have important advantages and disadvantages (Table 3). The characteristics of a specific cell type and the disease being studied may have an important influence on the researcher's choice of cellular model and, in some

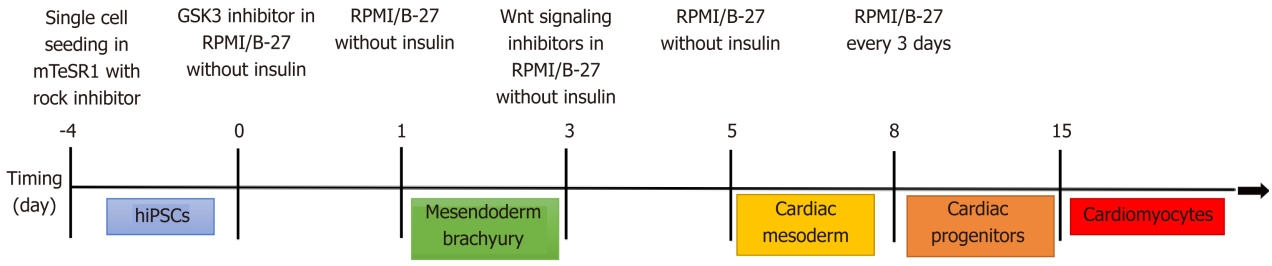

Figure 3 Cardiomyocyte differentiation protocol. Modified from Lian *et al*[135], 2012. hiPSCs: Human induced pluripotent stem cells.

situations, the study of both fibroblasts and iPSCs may be complementary. For instance, in a disease in which the interaction between cardiomyocytes and fibroblast plays a role in the development of the pathogenesis, for example in cardiac fibrosis and arrhythmias[127], the study of both cell types would likely be beneficial.

In order to solve the lifespan problem with primary cells such as fibroblasts, reversible immortalization could be performed to increase the number of passages and limit the risk for the development of aberrations in the genome[128]. In one study, this reversible immortalization was performed in primary neonatal rat cardiomyocytes using lentiviral transduction with either simian virus 40 large T antigen (TAg) or Bmi-1 together with the human telomerase reverse transcriptase (hTERT). After the cells were expanded, the introduced genes were removed using an adenoviral vector expressing Cre recombinase. The transduction of Bm1-1/hTERT into the primary cardiomyocytes successfully immortalized the cells and they maintained the expected cell morphology and presence of contact inhibition, suggesting that the cells had not become aberrant during the immortalization process[129]. This technique is an example of how genetic engineering could be used to overcome some of the limitations of cell biology which may be useful to researchers seeking to study a particular cell type.

Although patient-derived iPSCs and the differentiated cells that are created are excellent models of disease, the generation of appropriate controls is essential since they will help to define the abnormal phenotype. For some diseases that are enriched in specific populations with a unique genetic background, for example, DCMA, which is highly prevalent in the Hutterite population of southern Alberta[130], there is a need for controls who also have the same genetic background. The Hutterites are an isolated and genetically-closed population descended from a limited number of European ancestors with a communal religious lifestyle[131]. CRISPR/Cas9[132] can be used to repair the DNA mutation in patient cells to create isogenic controls[133] that are genetically identical except for a single genetic mutation background[134].

CONCLUSION

Cellular models represent an important tool for investigating rare human diseases including the genetic cardiomyopathies. Generic immortalized cells are the most commonly used cell model as they are the easiest to handle in terms of proliferation capacity, growth rate and low maintenance and can be easily genetically manipulated. Conversely, obtaining cells from individual patients allows the study of inter-individual differences and the important role of genetic modifiers in shaping disease phenotype and increases the possibility of developing personalized therapeutics. Certainly, *in vitro* models have some significant limitations but, in many cases, can provide a model that is otherwise not available. Particularly for cells differentiated from iPSCs, it is true that further research is necessary to optimize these cells but the potential for the development of an accurate and personalized cellular model is very promising for those diseases where conventional cells and animal models are limited.

Table 3 Advantages and disadvantages of different cell types for modeling disease *in vitro*

Properties	Fibroblasts	iPSCs
Proliferation capacity	+	++
Self-renewal	No	Yes
Longevity	Limited	Unlimited
Differentiation	No	Yes
Metabolism	Quiescent	Energetic
Acquisition	Easy	Difficult
Cost	+	+++
Ease of maintenance	Simple	Difficult
Necessary expertise	Low	High
Disease modeling	+	++
Structure	Single elongated cells	Round colonies/beating CM sheets
Maturation	Not applicable	Required for CM

iPSCs: Induced pluripotent stem cells; CM: Cardiomyopathy.

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ORIGINAL ARTICLE

Basic Study

Differential effects of atrial and brain natriuretic peptides on human pulmonary artery: An *in vitro* study

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Institutional review board statement: The study was reviewed and approved by the North West – Liverpool Central Research Ethics Committee (Approval no: 15/NW/0808).

Informed consent statement: All patients were consulted and consented for resected lung tissue to be studied for our research prior to their operation at the time of their consent for surgery.

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Abstract

BACKGROUND

The prevalence of cardiovascular diseases, especially heart failure, continues to rise worldwide. In heart failure, increasing levels of circulating atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) are associated with a worsening of heart failure and a poor prognosis.

AIM

To test whether a high concentration of BNP would inhibit relaxation to ANP.

METHODS

Pulmonary arteries were dissected from disease-free areas of lung resection, as well as pulmonary artery rings of internal diameter 2.5–3.5 mm and 2 mm long, were prepared. Pulmonary artery rings were mounted in a multiwire myograph, and a basal tension of 1.61gf was applied. After equilibration for 60 min, rings were pre-constricted with 11.21 μmol/L $PGF_{2\alpha}$ (EC_{80}), and concentration response curves were constructed to vasodilators by cumulative addition to the myograph chambers.

RESULTS

Although both ANP and BNP were found to vasodilate the pulmonary vessels, ANP is more potent than BNP. pEC50 of ANP and BNP were 8.96 ± 0.21 and 7.54 ± 0.18, respectively, and the maximum efficacy (E_{max}) for ANP and BNP was -2.03 gf and -0.24 gf, respectively. After addition of BNP, the E_{max} of ANP reduced from -0.96gf to -0.675gf (P = 0.28).

CONCLUSION

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BNP could be acting as a partial agonist in small human pulmonary arteries, and inhibits relaxation to ANP. Elevated levels of circulating BNP could be responsible for the worsening of decompensated heart failure. This finding could also explain the disappointing results seen in clinical trials of ANP and BNP analogues for the treatment of heart failure.

Key words: Heart failure; Atrial natriuretic peptide; Brain natriuretic peptide; *In-vitro*; Humans

Core tip: This study demonstrated that both atrial natriuretic peptide and brain natriuretic peptide (BNP) vasodilate isolated human pulmonary artery rings, and that BNP acts as a partial agonist and inhibits the effects of atrial natriuretic peptide. The finding that the addition of BNP inhibits the effects of atrial natriuretic peptide suggests that BNP does act as a partial agonist, and could be advancing the progression to decompensated heart failure.

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INTRODUCTION

Decompensated heart failure is a worldwide health issue that is associated with considerable morbidity and mortality[1,2]. Despite the development of several device- and medical-based therapies over the past few decades, the rate of rehospitalisation and early death has not significantly improved[3].

The natriuretic peptides (NPs) family consists of three structurally interrelated vasoactive peptides, and was initially discovered by de Bold *et al*[4] in 1981. The family includes atrial natriuretic peptide (ANP), brain natriuretic peptides (BNPs) and C-type natriuretic peptide (CNP), which are mainly secreted by cardiac myocytes in response to wall stress[5,6]. ANP and BNP act *via* guanylyl cyclase-linked natriuretic peptide receptor-A (NPR-A), whereas CNP activates the related cyclase natriuretic peptide receptor-B (NPR-B)[7]. ANP and BNP exert their beneficial effects by reducing systemic and pulmonary vascular resistance, and by increasing natriuresis and diuresis[8]. In addition to their haemodynamic effects, NPs attenuate vascular smooth muscle proliferation and cardiac hypertrophy[9,10]. They also inhibit the synthesis of growth factors, by counteracting the effects of the renin-angiotensin system, which is involved in the development of pulmonary hypertension[11].

In vitro studies on pulmonary arterial rings and isolated lung models have shown that ANP and BNP infusion induced pulmonary vasodilation by reducing pulmonary vascular resistance[12,13]. However, in heart failure, increasing levels of circulating ANP and BNP are associated with a worsening of heart failure and a poor prognosis[14]. The aim of this study is to evaluate whether BNP acts as a partial agonist, and inhibits the effects of ANP.

MATERIALS AND METHODS

Study patients

Local research ethics committee and institutional (Hull & East Yorkshire Hospitals NHS Trust) Research and Development Department approval was obtained for the use of lung specimens and surplus lung tissue from patients undergoing elective lobe or lung resection for cancer. Patients gave written consent for the use of surplus tissue for research purposes.

In accordance with the recommendations of the human tissue act (2004) 127 and the conditions of the local ethics committee approval, the donor patient was anonymous to the researcher.

Tissue collection

Excess segments of pulmonary artery were obtained from patients undergoing lobectomy, and the sample was immediately transferred to the lab in Krebs-Henseleit solution after resection. After the removal of connective tissue, the pulmonary artery (PA) sample was divided into 2 mm long rings. The small pulmonary vessels with an internal diameter of 2-4 mm were used for these experiments.

Experimental protocol

A multiwire myograph system was used for the measurement of isometric tension. Under physiological conditions (37°C, 21%O_2), PA rings were mounted in Krebs Henseleit solution. A resting tension of 1.61 gf was applied, which was calculated from earlier experiments[15], and the vessels were left to equilibrate for 60-90 min. After equilibration, vessels were pre-constricted with 11.21 μmol/L PGF2**α** ($EC_{80,}$ calculated from earlier experiments[16]), and concentration response curves were constructed to ANP and BNP by cumulative addition to the myograph chambers.

In another set of experiments, once the vessels tension reached a plateau after pre-constriction with PGF2α, 300 nmol/L of BNP was added and the vessels were left for 30 min. When a stable resting tension was achieved, concentration response curves were constructed to ANP. Vessels were then washed for 30 min, and the whole experiment was repeated again without the addition of BNP.

Active tension was calculated in gram force (gf) as maximum tension at plateau (gf) – resting tension (gf). The maximum efficacy (E_{max}) for each agent was determined in gf and expressed as gf/mm internal diameter of each vessel (to take into account the variability in PA ring diameter). The integrity of the endothelium was confirmed with 1μM Acetylcholine, and KCl was added to check viability. Vessels that did not constrict with KCl were excluded from the study. Figure 1 shows the schematic representation of myograph setup for measuring isometric tension.

Chemicals

A 5% CO_2/balance air was sourced from BOC Limited (Guilford, Surrey, United Kingdom). The agents used were (supplier in parentheses) ANP (Tocris Bioscience, part of Bio-Techne, Abingdon, United Kingdom), BNP (Tocris Bioscience) Acetylcholine (Sigma-Aldrich, St. Louis, MO, United States) and PGF2α (Tocris Bioscience). Stock solutions of drugs were prepared using the solvents recommended by the suppliers, and control responses to solvents were obtained when necessary. Fresh serial dilutions were made using the appropriate solvent for each experiment. All other reagents were obtained from Thermo Fisher Scientific unless otherwise stated.

Statistical analysis

Data are presented as mean ± SD, and n represents the number of individual PA rings used in an experiment. Agonist EC_{50} concentrations (the concentration required to elicit 50% of the maximum response) were determined using nonlinear regression to fit a standard slope model using the statistical analysis function of GraphPad Prism version 7.00 for Windows (GraphPad Software, La Jolla, CA, United States). More details can be found at http://www.graphpad.com/guides/prism/6/curve-fitting/index.htm). Agonist potency is presented as pEC_{50} (the negative logarithm of the molar EC_{50} concentration). Significance was taken as $P < 0.05$.

RESULTS

A total of 35 PA rings were obtained from 15 patients. The internal diameter of PAs ranged from 2.5–3.5 mm. Nine rings were not included, as they did not respond to KCl.

Concentration-dependent effect of ANP and BNP on human PAs

ANP and BNP caused a concentration-dependent relaxation of PAs pre-constricted to $PGF_{2α}$, with a pEC_{50} of 8.96 ± 0.21 and 7.54 ± 0.18 for ANP and BNP, respectively (Figure 2). The maximum efficacy (E_{max}) for ANP and BNP was -2.03 gf and -0.24 gf, respectively.

Another set of experiments was conducted to determine whether a high concentration of BNP would inhibit relaxation to ANP. After addition of BNP, the E_{max} of ANP was reduced by 30% from -0.96 gf to -0.675 gf ($P = 0.28$, $n = 11$) (Figure 3).

Concentration response curve of ANP-induced pulmonary vasodilation

All vessels vasodilate in response to ANP. Increasing the concentration of ANP from 3pmol/L–1 μmol/L were used on 8 PA rings. Maximal relaxation was seen at 100

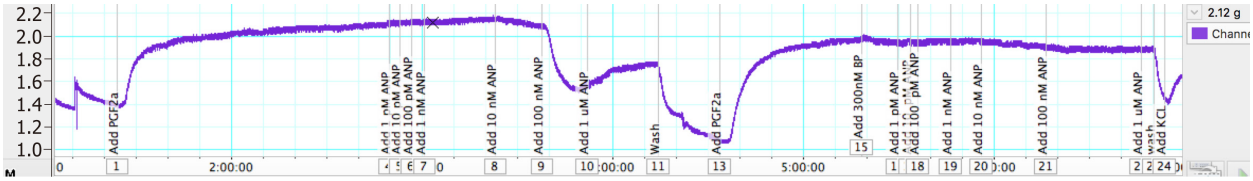

Figure 1 Schematic representation of myograph setup for measuring isometric tension.

nmol/L (log -7.0 mol/L), and the EC20, EC50 and EC80 were 0.17 nmol/L, 1.105 nmol/L and 7.01 nmol/L, respectively. The hill slope was -0.75 ± 0.5.

Concentration response curve of BNP-induced pulmonary vasodilation

In order to evaluate the effect of BNP on pulmonary vessels, 7 PA rings and a concentration of BNP from 1 nmol/L–1 μmol/L was used. As the concentration went above 10 nmol/L, vessels start to vasodilate and the maximum vasodilatory response was seen at 300 nmol/L (log -6.5 mol/L). The EC20, EC50 and EC80 were 13.3 nmol/L, 28.7 nmol/L and 61.5 nmol/L, respectively. The hill slope was -1.818 ± 2.55.

Cumulative concentration response curve of ANP and BNP-induced pulmonary vasodilation

In another set of experiments, the cumulative vasodilator effect of ANP and BNP on pulmonary vascular tone was investigated. Sixteen PA rings from seven patients, and an increasing concentration of ANP from 1 pmol/L–1 μmol/L, was used. Five rings were excluded, as they did not respond to KCl. When a stable resting tension was achieved, vessels were pre-constricted to 11.21 μmol/L $PGF_{2\alpha}$ (EC_{80}). When a stable plateau relaxation was achieved, the effect of ANP on active tension was determined by cumulative addition to the myograph chambers.

The PA rings were washed for 60 min, and were pre-constricted again with 11.21 μmol/L $PGF_{2\alpha}$ (EC_{80}). A single dose of 300 nm of BNP was added and left for 30 min. Once a plateau was achieved by cumulative addition to the myograph chambers, the concentration response curve of ANP was performed. The addition of BNP reduced the E_{max} of ANP by 30% (from -0.96 gf to -0.675 gf).

DISCUSSION

In this study, we demonstrated for the first time that (1) both ANP and BNP vasodilate isolated human PA rings; and (2) that BNP acts as a partial agonist and inhibits the effects of ANP. The finding that the addition of BNP inhibits the effects of ANP suggests that BNP does act as a partial agonist, and could be advancing the progression to decompensated heart failure.

The circulating concentration of ANP, BNP and CNP is low in healthy individuals, but it is elevated in heart failure patients, although to variable degrees (*e.g.*, CNP elevated to a lower extent than its counterparts)[17,18]. In patients with HF, circulating concentration of BNP exceeds that of ANP; this consistency of response and high dynamic range makes bioassays for plasma BNP more useful than ANP[19,20]. This might be due to the fact that BNP is also a marker of cardiac remodelling[21]. Previous studies have shown that in heart failure (HF) patients, BNP and NT-pro BNP (N-terminal pro b-type NP) are independent predictors of cardiovascular mortality, worsening HF and need for hospitalization[22-24]. Although BNP and NT-pro BNP have prognostic value, their therapeutic value is inconclusive in HF patients[25].

In the early 21st century, the United States Food and Drug Administration (FDA) approved the use of nesiritide (recombinant endogenous BNP) for heart failure patients[26]. However, several subsequent studies demonstrated that Nesiritide is associated with worsening renal function and increased risk of death[27]. A randomized, double blind, placebo-controlled, ASCEND-HF (Acute Study of Clinical Effectiveness of Nesiritide in Decompensated Heart Failure) trial concluded that nesiritide showed no substantial improvement in dyspnoea or clinical outcomes[28]. Another double-blinded, multicentre, randomized clinical trial, ROSE-AHF (Renal Optimization Strategies Evaluation - Acute Heart Failure) enrolled 360 patients. The study was designed to evaluate the use of low dose nesiritide, with the view that there would be less side effects and substantial therapeutic effects. However, the study failed to provide significant evidence in support of the routine use of nesiritide in heart failure patients[29].

Although NPs are always attractive therapeutic targets for heart failure treatment,

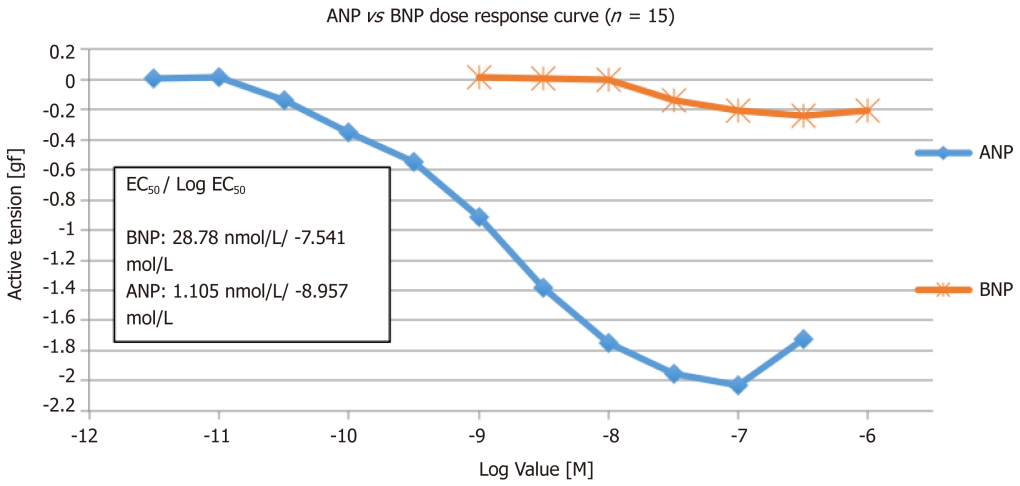

Figure 2 Cumulative concentration response curve to ANP and BNP (*n* = 15). Findings show that both ANP and BNP cause vasodilation. ANP is more potent and efficacious than BNP. pEC_{50} of ANP and BNP were 8.96 ± 0.21 and 7.54 ± 0.18, respectively. ANP: Atrial natriuretic peptide; BNP: Brain natriuretic peptide.

their use is limited by inadequate clinical efficacy. It is thought that the activity of neprilysin, a protease produced by the kidney that cleaves various vasoactive compounds including BNP, is increased in heart failure[30]. In heart failure, increasing levels of circulating ANP and BNP are associated with worsening heart failure and a poor prognosis. This raised the suspicion that BNP might act as a partial agonist and inhibit the effects of ANP, as shown in this study. These findings could also explain the disappointing results seen in clinical trials of ANP and BNP analogues for the treatment of heart failure. Further studies are needed to confirm the findings of this study, which raises the possibility that selective BNP antagonists could be of greater clinical benefit than BNP agonists for the treatment of heart failure.

Limitations

Our study had several limitations. It was a laboratory-based project that was carried out in a control setting, which may not truly reflect the *in vivo* environment. The therapeutic dose and the dose provided in the experiments may differ. We also used the pre-constrictor $PGF_{2\alpha,}$ and since the potency of the agent depends on the pre-constrictor, other pre-constrictors need to be analysed and compared. The full potential of the study needs to be backed by a double-blinded randomized control trial.

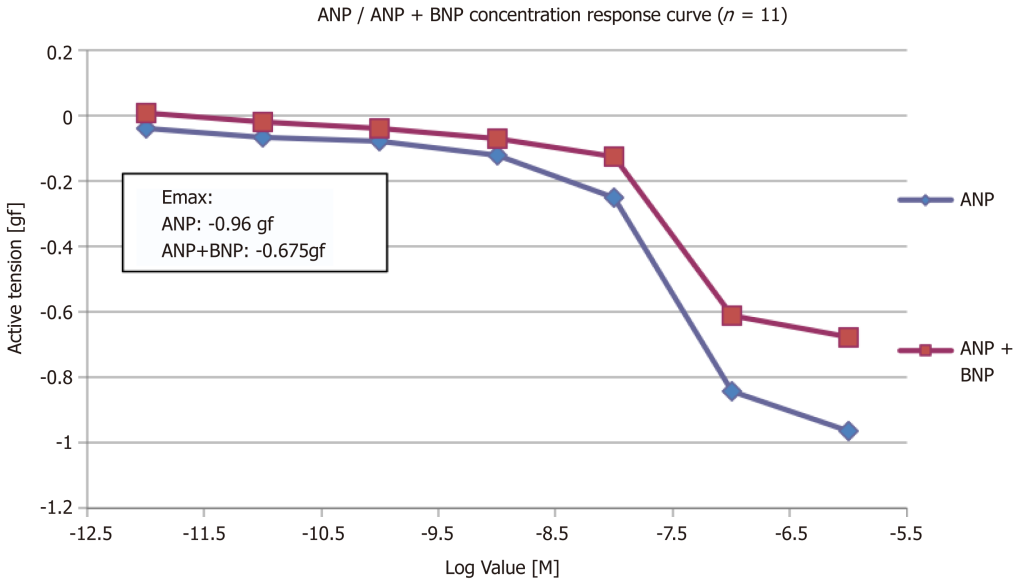

Figure 3 Concentration response curve to ANP alone and ANP + BNP (*n* = 11). The E_{max} of ANP was 0.96 gf, which reduced to -0.675 gf when BNP was added. ANP: Atrial natriuretic peptide; BNP: Brain natriuretic peptide.

ARTICLE HIGHLIGHTS

Research background

The prevalence of cardiovascular diseases, especially heart failure, continues to rise worldwide. In heart failure, increasing levels of circulating atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) are associated with worsening heart failure and poor prognosis.

Research motivation

ANP and BNP play an important role in homeostasis, but trials with BNP and ANP infusion showed disappointing results for unknown reasons.

Research objectives

The aim of this study was to evaluate whether BNP acts as a partial agonist and inhibits the effect of ANP.

Research methods

In this study, the effect of natriuretic peptides (ANP and BNP) on human pulmonary arteries was evaluated by cumulative addition to the myograph.

Research results

Both ANP and BNP act as pulmonary vasodilators, although ANP was found to be more potent and efficacious than BNP. Also, the addition of BNP reduced the efficacy of ANP.

Research conclusions

The study confirms that BNP inhibits the effects of ANP, and acts as a partial agonist. These findings also explained the disappointing results associated with the ANP and BNP infusion trials.

Research perspectives

Further studies are needed to validate the results of this study, and to evaluate the possibility of the clinical beneficial role of BNP antagonists for heart failure treatment.

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ORIGINAL ARTICLE

Basic Study

Evaluating the quality of evidence for diagnosing ischemic heart disease from verbal autopsy in Indonesia

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Abstract

BACKGROUND

Mortality and cause of death data are fundamental to health policy development. Civil Registration and Vital Statistics systems are the ideal data source, but the system is still under development in Indonesia. A national Sample Registration System (SRS) has provided nationally representative mortality data from 128 sub-districts since 2014. Verbal autopsy (VA) is used in the SRS to obtain causes of death. The quality of VA data must be evaluated as part of the SRS data quality assessment.

AIM

To assess the strength of evidence used in the assignment of Ischaemic Heart Disease (IHD) as causes of death from VA.

METHODS

The sample frame for this study is the 4,070 deaths that had IHD assigned as the underlying cause in the SRS 2016 database. From these, 400 cases were randomly selected. A data extraction form and data entry template were designed to collect relevant data about IHD from VA questionnaires. A standardised categorisation was designed to assess the strength of evidence used to infer IHD as a cause of death. A pilot test of 50 cases was carried out. IBM SPSS software was used in this study.

RESULTS

Strong evidence of IHD as a cause of death was assigned based on surgery for coronary heart disease, chest pain and two out of: sudden death, history of heart disease, medical diagnosis of heart disease, or terminal shortness of breath. More than half (53%) of the questionnaires contained strong evidence. For deaths

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outside health facilities, VA questionnaires for male deaths contained acceptable evidence in significantly higher proportions as compared to those for female deaths. ($P < 0.001$). Nearly half of all IHD deaths were concentrated in the 50-69 year age group (48.40%), and a further 36.10% were aged 70 years or more. Nearly two-thirds of the deceased were male (58.40%). Smoking behaviour was found in 44.11% of IHD deaths, but this figure was 73.82% among males.

CONCLUSION
More than half of the VA questionnaires from the study sample were found to contain strong evidence to infer IHD as the cause of death. Results from medical records such as electrocardiograms, coronary angiographies, and load tests could have improved the strength of evidence and contributed to IHD cause of death diagnosis.

Key words: Verbal autopsy; Data quality evaluation; Mortality; Cause of death

Core tip: In many countries in Southeast Asia, systems for recording mortality and causes of death are under development. In such settings, due to large proportions of deaths happening outside of health facilities, verbal autopsy interviews with families of the deceased are often used to ascertain the cause of death. However, there is a need to evaluate the quality of cause of death estimation from the verbal autopsy. This study specifically addresses the assignment of ischemic heart disease as a cause of death, concluding that a significant proportion of deaths were assigned this cause using strong evidence.

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INTRODUCTION

Sustained, accurate and timely data on mortality and cause of death patterns, especially the leading causes of death, is essential for local, national and global public health policy development, evaluation, and research[1,2]. The optimal method of obtaining data on mortality and causes of death is to have an attending physician complete a medical certificate of cause of death with the support of detailed clinical documents and to register these deaths in a universal Civil Registration and Vital Statistics (CRVS) system[3,4]. Efficient CRVS systems are still under development in many countries in the Asia-Pacific region, including Indonesia. As an interim step towards strengthening CRVS systems, Indonesia has established a national Sample Registration System (SRS)[5], similar to other countries in the region with large populations, such as India, China, and Bangladesh[6-8]. The aim of the Indonesian national SRS is to register deaths in a nationally representative sample of 128 sub-districts across the country and to estimate indicators of total and cause-specific mortality for health policy and program evaluation. Despite potential limitations in data availability, as well as quality since its inception in 2014, the Indonesian SRS has consistently reported ischemic heart disease (IHD) among the observed leading causes of death in the sample population from 2014-2016[9]. Therefore, it is important to evaluate the reliability of the Indonesian SRS in determining IHD as an underlying cause of death.

Since most deaths in Indonesia occur at home without medical attention, there is limited potential to implement medical certification of cause of death in the SRS. Under these circumstances, an alternative process termed verbal autopsy (VA) is used to ascertain the cause of death in the Indonesian SRS. VA involves a retrospective interview with the deceased's relatives or primary caregivers, who are familiar with the illness and circumstances preceding death[10]. The interview is carried out by trained interviewers after a certain interval following the death. The questionnaire

collects information about pre-existing disease suffered by the deceased, symptoms and clinical events during the illness preceding death, as well as details of interactions with health facilities before death, as reported by the respondent. Based on the answers from the interview, the cause of death is inferred through physician review of completed questionnaires, who then assign probable cause(s) for each death, or the cause of death is inferred using computerised algorithms[10]. The World Health Organi-zation (WHO) has recognised VA as a viable alternative for ascertaining causes of death for population health assessments, where medical certification of cause of death by attending physicians is not available, and has recommended international standards for this methodology[11]. As in Indonesia, there is now a movement in other settings with low-performing vital statistics systems to integrate VA into routine data collection[12].

The diagnoses from VA can be influenced by many factors, such as the design of questionnaires, interviewer skills, characteristics of respondents (including proximity to the deceased), recall period for the interview, and method used for ascertaining causes of death[13]. Given these potential sources of bias, the WHO has recommended the conduct of scientific studies to assess the quality of causes of death from VA[14,15]. Hence, this study was designed to evaluate the quality of evidence used to assign IHD as a cause of death from VA, in order to determine the reliability of IHD mortality estimates from the Indonesian SRS. The study also evaluated potential differences in the quality of evidence according to age group, gender, and place of death of the deceased, in order to develop recommendations to strengthen VA data quality from ongoing SRS operations.

MATERIALS AND METHODS

In general, the optimal method to validate VA methods is to compare the underlying cause of death derived from the VA to the reference diagnosis of the underlying cause for the same death, as derived from a pathological autopsy or the next best alternative, medical records for the deceased[16,17]. In view of the limited availability of pathological autopsies or medical records for community deaths in Indonesia, this study was designed to evaluate the quality of evidence that was available from VA questionnaires, to formulate the diagnosis of ischaemic heart disease as the underlying cause of death from VA. For this purpose, the study reviewed a sample of VA questionnaires for content related to IHD, in terms of medical history, symptoms, and signs of terminal illness, and details of clinical events and treatment as recorded in the questionnaire. VA data quality was analysed according to three categories of strong, acceptable, and weak evidence, a methodology that is conceptually similar to that used in studies to evaluate the quality of evidence for medical certification of causes of death[18-20].

Study design and sample

A cross-sectional study was designed to evaluate the quality of evidence recorded in a sample of VA questionnaires for which IHD was diagnosed as the underlying cause of death from the SRS in 2016. Overall, 30,633 deaths were registered in the 2016 SRS, of which 4,070 deaths had IHD assigned as the underlying cause of death, and this group forms the sampling frame for this study[9]. There was no prior information on the expected proportion of VA cases with strong evidence to support an IHD diagnosis. Hence, to maximise our sample size, we hypothesised that about 50% of VA questionnaires would have strong evidence, and it was estimated that a random sample of 384 questionnaires would be required to estimate this proportion at the 95% confidence level, within a 5% tolerable margin of error[21].

Data collection and processing

A total of 400 VA questionnaires with IHD as the underlying cause of death were randomly selected from the sampling frame. At first, the sample was tested and found to be representative of the whole sampling frame by age and sex. A data extraction form was used to collect required information of interest from the sampled VA questionnaires for the variables listed in Table 1. A brief explanation of the relevance of these variables for evaluating quality of evidence from VA will help place this study into context. In general, the variables presented in Table 1 were either used to assess the quality of evidence or to analyse the determinants or predictors of data quality. The place of death, whether at home or in the hospital, could influence the availability of specific information on the terminal illness, specifically with regard to the details of treatment and diagnosis. The relationship of the respondent could determine their proximity to the deceased, and therefore their knowledge of the terminal illness and events. The length of the recall period between the death and the

interview could also affect the quality of information. The past medical history and specific details of symptoms, signs and events during the terminal illness serve as primary data for reviewing physicians to formulate diagnoses.

The questionnaires also provide important information from three sections that record information in unstructured formats. Firstly, respondents describe their recollec-tions of the symptoms and clinical events during the terminal illness leading to death, in their own words. This section is referred to as the open narrative section. Secondly, the questionnaire also records information on the use of health services and any supporting information from other health records, such as hospital discharge state-ments, laboratory or imaging test reports, or drug prescriptions, among others. Finally, the questionnaire has a section in which the reviewing physician documents a summary of his impressions from the questionnaire review, which provides a basis for his/her assignment of causes of death. In some instances, this section could include information based on the reviewing physician's prior knowledge of the deceased and the terminal illness. Information from these three sections was trans-cribed and translated by local SRS staff, which were used in ascertaining quality of evidence.

The study team identified several key symptoms and other elements of information potentially available from VA interviews that could be used to diagnose IHD. The key symptoms include chest pain, terminal shortness of breath, and sudden death. History of heart disease in the deceased is also important evidence to support the diagnosis of IHD as the cause of death. A history of hypertension is also considered as a risk factor associated with cardiovascular disease mortality. A special variable termed "medical diagnosis" was created from the data, which was rated positive if either IHD was recorded as the cause of death reported by health staff for deaths in hospitals, or if IHD was recorded as a cause by the reviewing physician in the case summary. The study team developed three categories to assess the strength of evidence for the diagnosis of IHD, based on a combination of clinical history, symptoms and diagnostic information, as available. Each case was assigned a category of strength of evidence, the criteria for which are listed in Table 2.

Data analysis

Firstly, this study calculated the distribution of IHD deaths in the study sample by sex, age, place of death (inside or outside health facilities), previous medical history, and presence of risk factors. Data quality consistency between open narratives and structured questions in the questionnaire was measured as an indicator to assess the quality of the VA interview.

Next, this study calculated the frequency and proportion of cases assigned to each category of strength of evidence. Further, the distribution of the strength of evidence was evaluated by sex, age, place of death (hospital or home), the relationship of the respondent with the deceased, and whether the respondent resided with the deceased during the course of death. IBM SPSS statistical software was used in this study to calculate chi-square values and p-values to detect the statistical significance of variation in the strength of evidence by socio-demographic information, place of death and the relationship between respondents and the deceased.

The study data were also evaluated for consistency as an indicator of data quality. In general, the open narrative section is likely to include specific elements of information, such as the occurrence of chest pain, terminal shortness of breath, and previous history of heart disease or hypertension, all of which are also directly enquired by the structured questions. Consistency of such information across both the open-ended sections as well as the structured questions can reflect the quality of the interview, as well as justify the need for both sections in the questionnaire if the information is present only in one source. This study has examined this consistency by comparing information for key variables between the structured questions and open narratives in the same questionnaire.

Ethics consideration

Ethical approval for the overall SRS study has been obtained from the Indonesian Ministry of Health. The study proposed here also obtained ethical approval from both the Australia National University Human Research Ethics Committee (protocol number 2018/493) and the Ethics Board of NIHRD, Indonesia.

RESULTS

As mentioned in the Methods, the study sample was tested and found to be representative of the overall IHD deaths in the SRS 2016 data. Table 3 demonstrates that more than half of the deaths were among males (58.40%), and nearly half of all

Table 1 Data variables used for analysis of quality of evidence from verbal autopsy questionnaires

Data category	Data variables
General information of deceased	Age / sex
	Place of death (health facilities/home)
	Relationship with respondent
	Recall period of interview
Structured questions	Previous medical history (heart disease, stroke, hypertension, diabetes, *etc.*)
	Signs and symptoms of terminal illness
	Risk factors
	Use of health services
	Cause of death provided by health staff
Open sections	Respondent's free-flowing narrative of the course of illness and terminal events
	Information from available health records
	Physician reviewer's case summary

IHD deaths were concentrated in the 50-69 year age-group (48.40%), in approximately the same gender ratio, and a further 36.10% were aged 70 years or more. More than twice the number of deaths occurred at home than in a health facility, while male deaths were more likely to have occurred in health facilities than female deaths, which could have an influence on gender diffe-rentials in the quality of available evidence. Similarly, about half of all cases had a previous history of heart disease, again with males more likely to have such history compared to females. Among the risk factors of importance, about 40.35% of IHD deaths had a prior history of hypertension. Overall, 44.11% of the deceased had a positive history of smoking, but more importantly, almost three-fourths of the male IHD deaths had ever smoked. The average recall period for interviews was about four months, which is within the recommendations for VA.

Table 2 presents the distribution of IHD deaths according to the various categories of strength of diagnostic evidence. Only 4 cases mentioned a previous history of cardiac surgery associated with terminal cardiac symptoms, which represented the strongest possible evidence for IHD. In addition to these four cases, a substantial number of questionnaires included definitive information on terminal chest pain along with other symptoms, positive history, or a medical diagnosis of IHD, as defined in the Methods section. Together, these cases accounted for more than half the sample being assigned to the category of strong diagnostic evidence for IHD from VA.

In another 22% of cases, there was evidence that was reasonably suggestive of IHD, either in the form of terminal chest pain, or a combination of history of sudden death with previous heart disease or a medical diagnosis. While less convincing than the criteria defined for the category of strong evidence, we chose to allocate these cases to the "medium" strength of evidence category. For the remaining cases, the VA questionnaires only included minimal information either in the form of isolated clinical features such as sudden death, terminal shortness of breath, or previous history of heart disease or hypertension. In all these cases, the questionnaires did not contain any information suggestive of any other potential cause of death, but the absence of specific evidence of IHD necessitates these cases to be assigned the category of weak evidence. In two of the sampled cases, there was no symptom suggestive of any cause of death and were hence clearly incorrectly assigned to be caused by IHD.

We further analysed the data to evaluate the demographic and other factors that could be associated with the strength of evidence for the diagnosis of IHD. For this analysis, we combined the deaths from "strong" and "medium" evidence into one category termed "acceptable" evidence and compared it with those from the "weak" evidence category, as shown in Table 4. The analysis showed that while there was no association between strength of evidence and age at death, acceptable evidence to diagnose IHD was significantly associated with the occurrence of deaths in hospitals. Acceptable evidence was also positively associated with deaths in males as compared to deaths in females (Table 4), but a stratified analysis (Table 5) showed that this association was statistically significant only for male deaths that occurred at home (P = 0.005). More pertinent was the finding that there was a significant likelihood of recording better evidence if the respondent belonged to the same generation as the deceased (spouse or sibling), as compared to either a parent or offspring of the deceased being from a different generation. Paradoxically, acceptable evidence was

Table 2 Distribution of each category of strength of evidence

Category	Criteria	Cases	Proportion
Strong	(1) Surgery for coronary heart disease (1%); (2) Terminal chest pain and two of: (A) Sudden death; (B) History of heart disease; (C) Medical diagnosis of heart disease[3]; (D) Terminal shortness of breath.	213	53%
Medium	(1) Terminal chest pain alone; (2) Sudden death AND either: (A) History of heart disease OR; (B) Medical diagnosis of heart disease; (3) Only medical diagnosis of heart disease.	87	22%
Weak	(1) Only history of heart disease; (2) Only symptomatic evidence (without chest pain): (A) Sudden death; (B) Hypertension; (C) Shortness of breath.	98	24.5%
Nil	No evidence for the cause of death.	2	0.5%
TOTAL		400	100

Medical diagnosis of heart disease[3]: (A) informed by health facility staff where treatment accessed during illness; or (B) recorded by local health centre physician with prior knowledge of medical condition of deceased.

significantly associated with longer recall periods (> 90 d), a finding that was also observed in the same population for a similar study conducted to assess the quality of evidence for VA diagnoses of cerebrovascular disease.

This study also analysed the availability and consistency of information across different sections of VA questionnaires. Figure 1 displays the frequencies of positive responses to several key variables from either or both the structured questions and open text sections of the questionnaire. Overall, there was the consistency of information across the two sources within the questionnaire in only 60%-70% of deaths for all of the key variables. The symptoms of chest pain, sudden death, and previous history of heart disease and hypertension were all reported more frequently in response to structured questions. In contrast, the symptoms of shortness of breath and unconsciousness were reported more often in the open text sections. A positive response in at least one source was used in assigning the category of strength of supporting evidence for each case.

Another factor that could influence the quality of information in the VA questionnaires is whether the death took place in a health facility or at home. Figure 2 displays the proportions of deaths in these two locations for which a positive response was provided for certain key symptoms, as well as for the constructed variable "medical diagnosis of heart disease" (see Methods). As per usual expectation, respondents for deaths in health facilities provide higher levels of positive responses, indicative of increased awareness of the clinical features of the terminal illness, likely communicated by the health care staff. This is also evident in the higher proportions of cases with a medical diagnosis of heart disease, as recorded in the questionnaire. All of these observations support the general finding of significantly higher levels of acceptable evidence for deaths in hospitals, as reported in Table 4.

DISCUSSION

VA is currently being promoted as a viable option for deriving information on causes of death in countries where medical certification of cause of death is unavailable or limited[21]. Despite methodological limitations of VA in terms of its reliance on second-hand information from the deceased's relatives, which follows a considerable recall period, causes of death from VA are increasingly being used for national mortality estimation[22,23]. IHD is estimated to be among the top five leading causes of death in the world, as well as in Indonesia. To our knowledge, this study provides the first ever empirical assessment of the quality of evidence available to infer a diagnosis of ischaemic heart disease as the underlying cause of death from VA. Our study identified that more than half (53%) of sample questionnaires from the Indonesian SRS contained strong evidence about IHD. Furthermore, another 22% of cases included sufficient evidence to support a diagnosis of IHD. For the remaining one

Table 3 Sample description by socio-demographic factors and health background

Variable	Female		Male		Total		Chi-square	*P* value
	n	%	*n*	%	*n*	%	χ^2	*P*
Age								
< 30	2	1.2	3	1.3	5	1.3	-	-
30-49	27	16.3	30	12.9	57	14.3	-	1.000
50-69	70	42.2	123	52.8	193	48.4	-	1.000
70+	67	40.4	77	33.0	144	36.1	-	1.000
All ages	166	41.6	233	58.4	399	100		
Place of death								
Health facilities[1]	46	28.0	80	34.3	126	31.6	-	-
Home[2]	118	72.0	153	65.7	271	67.9	1.8	0.185
Medical history								
Hypertension	74	44.6	87	37.3	161	40.4	-	-
Heart disease	73	44.0	133	57.9	206	51.6	4.2	0.041[a]
Diabetes	20	12.0	30	12.1	50	12.5	-	0.516
Risk factors								
Smoking	4	66.7	172	93.0	176	44.1	-	-
Alcohol	2	33.3	13	7.0	15	3.8	-	0.072
Recall period in days								
Mean	110		123				-	-
Median	102		114				0	0.998

[1]Health facilities[1]: Includes deaths occurring in hospital, other health facilities and walk-in clinic; Home[2]: Includes deaths occurring at home and in transit;
[a]*P* value < 0.05.

fourth of the sample, although the evidence used to assign IHD was weak, there was no evidence in the questionnaires to indicate an alternative VA-based cause of death. Overall, our study findings indicate that VA protocols employed in the Indonesian SRS generate evidence of sufficient quality for diagnosing IHD as an underlying cause of death, but with some room for improvement.

More detailed analyses identified that there was a significant likelihood for acceptable diagnostic evidence of IHD from VA for deaths that occurred in health facilities, among male deaths at home, or for which the respondents were from the same generation as the deceased. The availability of strong evidence for hospital deaths is generally plausible and readily understood, owing to the potential for family members to receive direct medical information about the illness from medical staff, which was observed for both male and female deaths. However, for deaths at home, there was a significantly higher proportion of male deaths with acceptable evidence compared to female deaths. In general, it was also observed that the quality of evidence was uniformly better from wives as respondents, in comparison with husbands as respondents (data not shown). This could be a reason for the gender differentials in the quality of evidence. More detailed qualitative research is required to ascertain the reasons for this difference in response patterns. Also, the finding that respon-dents from the same generation (either a spouse or sibling) as the deceased are associated with better quality of VA evidence as compared to either the parents or offspring (a different generation) of the deceased is important. This finding suggests that for adult deaths, interviewers should actively seek and preferably conduct the VA with a spouse or sibling, rather than other adult relatives who may not pay the same attention to details of the terminal illness or the health care received by the deceased.

From a diagnostic perspective, IHD poses particular challenges, in that its cardinal symptom - acute chest pain - is essentially subjective in nature, as compared to the directly observable unilateral paralysis in cases of cerebrovascular stroke. The subjective nature of the occurrence, intensity, and duration of chest pain makes it challenging for VA respondents to report this symptom, as evidenced from its reporting in only about 60% of deaths. This is further compounded by the incidence of

Table 4 Associations between strength of evidence and Verbal Autopsy interview characteristics

Variable	Category	Evidence				Chi-square	*P* value
		Acceptable		Weak			
		n	%	*n*	%	X^2	*P*
Sex of deceased							
	Male	189	81.5	43	18.5	-	-
	Female	110	66.7	55	33.3	11.4	< .001[a]
Age of deceased							
	30-69	191	76.4	59	23.6	-	-
	70+	106	74.1	37	25.9	0.3	0.627
Place of death							
	Hospital	111	87.4	16	12.6	-	-
	Home	188	69.9	81	30.1	14.3	< 0.001[a]
Relationship between respondent and deceased							
	Spouse/sibling	109	86.5	17	13.5	-	-
	Parent/offspring	126	69.6	55	30.4	11.8	< 0.001[a]
Respondent living with the deceased							
	Yes	223	76.4	69	23.6	-	-
	No	69	70.4	29	29.6	0.2	0.281
Recall period							
	> 90 d	172	80.0	43	20.0	-	-
	≤ 90 d	122	70.1	52	29.9	4.4	0.036[a]

[a]*P* value < 0.05.

sudden death in IHD, which is mostly due to cardiac causes as compared to cerebrovascular stroke[24]. In the SRS VA protocol, the structured question on 'sudden death' enquires about the occurrence of death in an individual without any serious illness in the period immediately preceding 24 h. The response to this question also has a degree of subjectivity, which gets further blurred by the length of the recall period. Also, there needs to be clarity in the interviewer's understanding of the phenomenon of sudden death, and (s)he should be able to clearly convey this concept to the respondent, in order to elicit and record the correct response. In our study sample, sudden death was reported in over 70% of cases. In the absence of a medical certificate of cause of death, we considered that a verbal confirmation of chest pain and sudden death is highly suggestive of the cause being IHD. A third important element in our diagnostic criteria was the availability of a "medical diagnosis", as defined in the methods. The SRS interview protocol gives strict instruction to interviewers to not ask leading questions naming specific causes when recording the open narrative, or the structured questions on health care access, or diagnoses provided by healthcare staff. Further, a diagnosis of IHD recorded in the VA reviewing physician's summary is either based on his opinion from the questionnaire review or from prior knowledge of the deceased's clinical history. Hence, taken together, these three elements - chest pain, sudden death, and a medical diagnosis - formed the core criteria for strong evidence in support of an IHD diagnosis. Other categories of evidence included less specific features for IHD.

In terms of the availability of direct clinical information, only four cases reported a previous history of cardiac surgery. Also, there was no information from the health records section providing diagnostic evidence from previous hospital discharge documents, electrocardiograms, laboratory or imaging test reports, or drug prescriptions, which could have aided us in evaluating the strength of evidence. Such information was not available, even though a third of the study sample deaths occurred in hospitals, for which the only useful information from the health records section was from the cause of death communicated by the health staff. A recent study in Vietnam identified that clinical discharge records are valuable evidence for deaths in individuals who accessed health facilities but died within a month following discharge[25]. A likely reason for the absence of this information in Indonesia is the

Table 5 Deaths in health facilities and outside health facilities by gender

Deaths in health facilities	Acceptable evidence		Weak evidence		Chi-square	
	n	%	*n*	%	χ^2	*P* value
Male	72	90.0	8	10.0	-	-
Female	38	82.6	8	17.4	-	0.271
Deaths outside health facilities						
Male	117	77.0	35	23.0	-	-
Female	71	60.7	46	39.3	-	0.005[a]

[a]*P* value < 0.05.

cultural practice of disposing all medical documents and health care materials belonging to the deceased at the time of or soon after the funeral. Future community sensitization events about the VA program could appeal for such documentation to be preserved and made available during VA enquiries.

The findings on the availability and consistency of information from different sections of the VA questionnaire also have important implications for VA implementation. The two main questionnaire components comprising the open text sections and structured questions offer opportunities to record similar information for certain key variables potentially. This provision has been made in the questionnaires to accommodate an observed inherent variability in response patterns during VA interviews, as demonstrated both in our study (Figure 1) as well as in a similar study that only assessed such variation in the reporting of paralysis in deaths from cerebrovascular stroke in Vietnam[26]. Some respondents require prompting through structured questions to elicit all relevant information, while others are more comfortable with giving information in their own words and are non-committal or even inattentive during the structured questions. The open narrative section in questionnaires has generally been found to be very important in determining the cause of death, similar to studies conducted elsewhere[27,28]. Constructing a timeline that puts the history of disease, individual symptoms, signs, and chronology of clinical events together can help characterise the events leading to death, if the respondents were familiar with the deceased. In the Indonesian VA physician review protocol, reviewers are also trained to utilise the information from all sections of the questionnaire to construct such a summary, in order to guide their diagnostic decisions. In many instances, it is likely that physician reviewers would be able to diagnose causes of death largely from the open narratives, although they should always seek corroborating information from the structured questions. Ultimately, better consistency of information across both sources increases confidence in the veracity of information available to formulate diagnoses.

In conclusion, this study demonstrates a process for reviewing the quality of evidence in VA questionnaires, in the context of assigning ischaemic heart disease as the underlying cause of death. While acceptable evidence was available for three-fourths of the cases in our study sample, several measures could be taken to improve overall data quality. Firstly, the questionnaire could be modified to elicit more detail in regard to the designation and/or qualification of health staff (doctors, nurses, or paramedics) who provided an opinion as to the cause of death for events in health facilities. This would enable more accurate use of this information in deciding the level of evidence. Secondly, communities should be sensitised to the benefit of retaining and sharing available health care documents within the household with the local health centre staff, instead of casual disposal following the final rites. Thirdly, the SRS program could initiate activities to liaise with secondary and tertiary hospitals in cities and major towns in the proximity of SRS sites, from where some cause of death-related data may be obtained for facility deaths. Eventually, medical certification of the cause of death scheme could be introduced in these hospitals, to improve the overall quality of evidence for mortality statistics from the SRS. Also, further qualitative research could help design improved community interactions to access the most appropriate respondent, as well as improved interviewing techniques to strengthen VA data quality. These study methods for ischaemic heart disease could be used as a model to investigate the quality of evidence for other major causes of death such as cerebrovascular disease, diabetes, tuberculosis, and chronic lung disease, among others, in Indonesia as well as other settings where VA is routinely implemented. Periodic evaluation of the quality of VA evidence is essential to

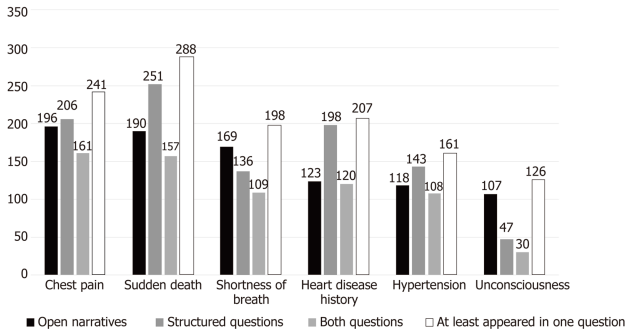

Figure 1 Quality consistency of data in different sections of questionnaire.

improve the empirical use of VA data for mortality and cause of death measurements for health policy, monitoring, and evaluation.

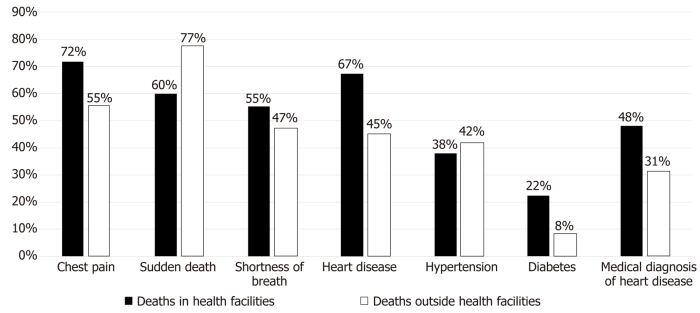

Figure 2 Symptom frequencies between deaths at health facilities and at home.

ARTICLE HIGHLIGHTS

Research background

Mortality and cause of death data are the basis for health policy and research. The Civil Registration and Vital Statistics (CRVS) system is the ideal source of data, but the CRVS in Indonesia is still under development. Since 2014, the National Sample Registration System (SRS) has provided nationally representative mortality data from 128 sub-districts. Verbal autopsy (VA) is used in SRS to obtain the cause of death.

Research motivation

The evidence available from the VA to diagnose causes of death must be assessed to establish the reliability and utility of SRS data. The diagnosis of VA may be influenced by many factors, such as questionnaire design, interviewer skills, characteristics of respondents (including proximity to the deceased), recall period for interviews, and methods for determining the cause of death. Given these potential sources of bias, the World Health Organization recommends conducting scientific research to assess the quality of VA's cause of death, hence necessitating this study.

Research objectives

This study was designed to assess the quality of evidence used to diagnose Ischaemic Heart Disease (IHD) as a cause of death from VA. The study also sought to evaluate various factors that could influence the quality of evidence, such as age and gender of the deceased, place of death, relationship of the respondent, and recall period.

Research methods

The study sample comprised a random sample of 400 deaths out of a total of 4,070 cases diagnosed from IHD in the SRS data for 2016. A data extraction form and data entry template were designed to collect relevant IHD data from VA questionnaires. A standardised classification was designed to IHD cases to categories with strong, medium and weak evidence. Strong evidence of IHD was defined to include surgery for coronary heart disease, or the history of chest pain along with two additional characteristics among sudden death; history of heart disease; the medical diagnosis of heart disease; or terminal shortness of breath. Statistical analysis was conducted to assess the frequency of cases with different levels of evidence, as well as to identify associations between case characteristics and levels of evidence.

Research results

Nearly half of all IHD deaths were concentrated in the 50-69 age group (48.40%), and another 36.10% were 70-years-old or older. Two-thirds of the deceased were male (58.40%). VA questionnaires for about three-quarters of all cases contained strong or medium evidence to diagnose IHD. Quality of evidence was significantly associated with the occurrence of deaths in hospitals, with male deaths at home, and with deaths for which the respondent belonged to the same generation as the deceased.

Research conclusions

VA diagnoses of IHD was found to be based on acceptable evidence in the majority of cases in the study sample. Attention is required to improve recording of information during VA interviews, particularly in regard to correct interpretation of responses for symptoms and signs, and more importantly, clinical details from interactions with health services. Such studies should be conducted for other leading causes of death in Indonesia, as well as across space and time.

Research perspectives

The study assessed levels and determinants of the quality of diagnostic evidence to assign Ischaemic Heart Disease as a cause of death from VA methods in Indonesia. The study results provided perspectives on VA data collection processes, evidence patterns guiding VA diagnosis, and the influence of various circumstances of the death event and household interview on the overall quality of evidence from VA.

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