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Clinical Medicine Insights: Cardiology

Echocardiographic Assessment of Mitral Valve Regurgitation, Pattern and Prevalence, Expanding Clinical Awareness Through an Institutional Survey with the Perspective of a Quality Improvement Project

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ABSTRACT

BACKGROUND: Mitral regurgitation (MR) is frequently reported in everyday echocardiograms; accurate assessment is essential for appropriate management and decision making.

OBJECTIVE: We performed a self-audit in order to define the prevalence and pattern of MR and to evaluate methods of assessment with the perspective of developing a quality improvement project.

METHODS AND SETTING: This retrospective analytical study was conducted in a university hospital. Inclusion criteria: age more than 18 years and medical records available within the facility, including a "complete" medical history. Using the picture archiving and communication system, we reviewed 961 echocardiograms performed over a 6-month period. The methods of assessment of native mitral valve regurgitation were reported, and also relevant medical data were collected using an electronic archiving system.

RESULTS AND DISCUSSION: Among the 961 patients reviewed, 322 (33.50%) had MR, with variable grades. MR pattern (organic versus functional) was not specified in 49.68% of cases. "Eyeball" assessment and "color jet area" were the most frequently used methods for MR assessment (90.06% and 27.95%, respectively), while "vena contracta" and "flow convergence" methods were rarely implemented (1.55% and 2.17%, respectively). Discussion is made according to current guidelines, while showing the strengths and weaknesses of each method.

CONCLUSION: The prevalence of MR was 33.50%, and in nearly half of cases, the MR pattern was not specified. Qualitative and semi-quantitative methods of assessment were mostly used; quantitative assessment should be implemented more frequently, in accordance with current guidelines. Increasing clinical awareness by creating and implementing a quality improvement project is essential in this context.

KEYWORDS: echocardiography, mitral regurgitation, assessment, quality, improvement

CITATION: Kossaify and Akiki. Echocardiographic Assessment of Mitral Valve Regurgitation, Pattern and Prevalence, Expanding Clinical Awareness Through an Institutional Survey with the Perspective of a Quality Improvement Project. *Clinical Medicine Insights: Cardiology* 2014:8 71–77 doi: 10.4137/CMC.S17367.

RECEIVED: May 28, 2014. RESUBMITTED: July 9, 2014. ACCEPTED FOR PUBLICATION: July 15, 2014.

ACADEMIC EDITOR: Thomas E Vanhecke, Editor in Chief

TYPE: Original Research

FUNDING: Authors disclose no funding sources.

COMPETING INTERESTS: Authors disclose no potential conflicts of interest.

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Background

Mitral regurgitation (MR) is increasingly prevalent. It is the second most common valve disease after aortic stenosis in Western countries, representing 32% of native left-sided valve disease¹; moreover, nearly 10% of people over 75-years old have significant MR.² In addition, the concept of physiological MR has been previously reported,³ and it may be encountered in 20–40% of subjects with structurally normal hearts. The high prevalence of MR is related to increased longevity, along with the advent of more sophisticated diagnostic techniques. Appropriate management of MR is highly dependent on an accurate diagnosis, and echocardiography is the cornerstone diagnostic test in this regard.⁴ According to recently published guidelines,^{5,6} a quantitative echocardiographic assessment of any "more than mild" MR is mandatory in order to yield an accurate diagnosis of severity and better management. In this study, we sought to evaluate MR pattern and prevalence, using institutional registries and archives, and to perform a self-assessment of the echocardiographic techniques used, with the aim of developing a quality improvement program.

Methods

Records of 1111 consecutive patients at The Centre Hospitalier Universitaire Notre Dame de Secours, Byblos, Lebanon, were retrospectively reviewed over a 6-month period from October 2013 until March 2014, using an electronic registry to retrieve patients' names, dates, and unit caseload. In addition, we used the "picture archiving and communication system (PACS)" to retrieve echocardiographic reports and images. Medical records were reviewed and relevant data recorded as appropriate, including medical history, referring physician, and echocardiography indication when available. Inclusion criteria consisted of age >18 years, medical record available within the facility including a "complete" medical history, and transthoracic echocardiogram performed in the facility within the study allocated time interval. Exclusion criteria consisted of prosthetic mitral valve (MV) or any previous interventional procedure on the MV, moderate or severe aortic valve disease, atrial fibrillation, and transesophageal echograms. Among the 1111 medical records, 961 were eligible for the study, and were divided into two groups (MR group and no MR group).

All echocardiograms were performed by experienced sonographers using a commercially available echocardiograph (Philips iE33 ultrasound system, Philips Medical Systems, Andover, MA), with 3.5 MHz transducer. Three-dimensional echo and deformation imaging were not available. All images were recorded digitally with the capacity to be analyzed offline. All echographic data were used unaltered from the original echocardiographic reports and PACS via electronic transfer. The present study protocol was approved by institutional research board and was carried out in accordance with the Declaration of Helsinki.

Statistical Analysis

Analysis was performed using the Statistical Package for the Social Sciences software, New York, USA (IBM). Data were expressed as mean \pm standard deviation, or number and percentage, as appropriate. Patients' characteristics between the two groups (MR group and no MR group) were analyzed with the Student's *t*-test for continuous variables and with Chi-squared test for categorical variables. Only univariate analysis was performed, as searching for independent correlates with MR was beyond the scope of the study. Prevalence rate of MR was calculated as the rate of MR in the studied population. A *P* value less than 0.05 was considered statistically significant.

Results

Among the 961 eligible patients, 611 (63.58%) patients were referred by cardiologists and the remaining 350 (36.42%)

patients were referred by non-cardiologists. There were 205 (21.33%) patients whose echocardiograms were performed on an outpatient basis. Only 504 patients (52.44%) had their echocardiogram indication clearly marked by means of the request form sheet. Among the 961 patients, there were a total of 322 patients with MR (mean age 75 \pm 9.5 years), yielding a MR prevalence of 33.50% in the studied population. Table 1 shows patients' characteristics.

All cases of MR were graded with regard to severity; mild MR was encountered in 193 (59.93%) patients, moderate MR in 105 (32.60%) patients, and severe MR in 24 (7.45%) patients. However, the categorization of a specific pattern (organic versus functional) was poorly provided, and there were 160 (49.68%) patients in whom MR pattern was not specified. The pattern of MR is shown in Table 2.

Specific etiologic or mechanistic data in each MR pattern (eg, degenerative or rheumatic in organic MR, ischemic or dilated cardiomyopathy in functional MR, etc.) were not often provided in reports. Table 3 shows how often different variables were described or listed in the reports, whether listed as normal or abnormal. Of note, leaflet morphology data (thickness, calcifications, etc.) were provided in 20.8% of cases, and leaflet dynamics (motion, tenting, tethering, etc.) were provided in 13.97%. Annular description (qualitative or quantitative) was provided in 55.90% of cases and annular dilatation was reported in 34.5%, jet characteristics (central, eccentric, multiple, etc.) were provided in 89.13%, left ventricle (LV) sphericity index in 17.7%, and MV prolapse (MVP) was reported in 4.5%.

"Eyeball" assessment was theoretically used in all patients; however, this qualitative and subjective method was cited in the records of 290 (90.06%) patients. "Color flow imaging," a semi-quantitative method using the ratio of jet area to left atrial area, was used in only 27.95%, whereas more quantitative methods such as vena contracta width and flow convergence were rarely implemented (1.55% and 2.17%, respectively). Other complementary methods (ie, continuity equation using volumetric aortic flow, spectral continuous

Table 1. Patient characteristics.

TOTAL (961 PATIENTS)	MR (322)	NO MR (639)	P VALUE
Age, years	75±9.5	59±7.3	<0.0001
Male gender	189 (58.69)	401 (62.75)	0.222
Tobacco use	201 (62.42)	309 (48.35)	<0.0001
Diabetes	55 (17.08)	76 (11.89)	0.027
History of RF	7 (2.17)	1 (0.15)	0.001
History of IE	5 (1.55)	0 (0)	0.001
Aortic valve disease	55 (17.08)	15 (2.34)	<0.0001
Hypertension	155 (48.13)	101 (15.80)	<0.0001
Coronary artery disease	97 (30.12)	79 (12.36)	<0.0001
Heart failure	59 (18.32)	45 (7.04)	<0.0001

Notes: Data are expressed as number (percentage) or mean \pm SD. Abbreviations: RF, rheumatic fever; IE, infective endocarditis.

Table 2. Pattern of MR.

MR PATTERN, n (%)	MILD 193 (59.93)	MODERATE 105 (32.60)	SEVERE 24 (7.45)		
Organic, 20 (6.21)	13	6	1		
Functional, 129 (40.06)	91	29	9		
Mixed, 13 (4.03)	8	4	1		
Non specified, 160 (49.68	3) 81	66	13		
Note: Results are expressed as numbers of MR in each pattern.					

Doppler density, and pulmonary flow reversal) were not used at all. Of note, the use of an integrated approach (qualitative + quantitative \pm complementary methods) was not reported. Moreover, there was no specific algorithm applied regarding the sequential use of methods according to MR complexity or severity. Table 4 summarizes the rate of implementation of the different diagnostic methods.

Discussion

Patient characteristics. Table 1 shows patients' characteristics of the studied population. The mean age in the "MR group" was significantly higher than that of the "no MR group," a logical finding given that many causal factors of MR increase with aging (eg, degenerative disease, coronary artery disease, heart failure, diabetes, and hypertension). Similarly, these conditions, when taken separately through univariate analysis, showed higher prevalence in the "MR group" (except male gender). Multivariate analysis was not performed in this setting, given that a search for correlates with MR was beyond the purpose of the study. Also, these factors are already established as correlates of organic MR (ie, infective endocarditis, rheumatic fever, degenerative

Table 3. Reported echocardiographic data relevant to MR.

disease, etc.) or functional MR (ie, ischemic cardiomyopathy, dilated cardiomyopathy, etc.).7

MR prevalence. MR prevalence in the general population ranges from 20% to 40%, and grading varies from trivial (or physiological), to mild, moderate, and severe.⁸⁻¹⁰ Nkomo et al. reported that more than 10% of people above 75 years old have significant (moderate to severe) MR.²

In the present study, we found a prevalence of MR of 33.50%, which is within the range reported in the literature. "Eyeball" and "color Doppler jet" methods were mostly used, and while these are valid approaches for MR diagnosis, such qualitative and semi-quantitative methods are not sufficient for grading MR.5 The concept of "physiological" MR is not widely recognized, and the criteria of its definition and diagnosis are still controversial.³ However, the existence of "less than mild" MR, with central minimal jet, without myocardial disease, and without identifiable MV apparatus abnormalities, validates the labeling of a minimal MR as "physiological."9,10 The percentage of "physiological" MR can range up to 66% in a general population older than 30 years.¹⁰ In addition, the frequency of MR is dependent on operator experience, methods used, and equipment sensitivity.¹¹ In this study, there was no mention of physiological MR, and we therefore assume that all minimal or "less than mild" MR, when detected, were labeled as mild MR.

MR pattern: organic versus functional. For an accurate assessment of MR, an extensive knowledge of the MV anatomy is mandatory, including valve leaflets and their respective segmentation (scallops; A1, A2, A3/P1, P2, and P3), commissures, annulus, chordae, and papillary muscles; also, a pertinent assessment of the LV, left atrium, and pulmonary pressure is essential. Moreover, a comprehensive knowledge of the pathoanatomic classification, as presented

MILD	MODERATE	SEVERE	TOTAL
193 (59.93)	105 (32.60)	24 (7.45)	
35 (18.13)	25 (23.80)	7 (29.16)	67 (20.80)
21 (10.88)	19 (18.09)	5 (20.83)	45 (13.97)
70 (36.26)	90 (85.71)	20 (83.33)	180 (55.90)
7 (3.62)	5 (4.76)	1 (4.16)	13 (4.03)
0	0	0	0 (0)
165 (85.49)	100 (95.23)	22 (91.66)	287 (89.13)
193 (100)	105 (100)	24 (100)	322 (100)
89 (46.11)	65 (61.90)	11 (45.83)	165 (51.24)
193 (100)	105 (100)	24 (100)	322 (100)
193 (100)	105 (100)	24 (100)	322 (100)
33 (17.09)	15 (14.28)	9 (37.5)	57 (17.70)
	MILD 193 (59.93) 35 (18.13) 21 (10.88) 70 (36.26) 7 (3.62) 0 165 (85.49) 9 193 (100) 89 (46.11) 193 (100) 193 (100) 33 (17.09)	MILD 193 (59.93) MODERATE 105 (32.60) 35 (18.13) 25 (23.80) 21 (10.88) 19 (18.09) 70 (36.26) 90 (85.71) 7 (3.62) 5 (4.76) 0 0 165 (85.49) 100 (95.23) 193 (100) 105 (100) 89 (46.11) 65 (61.90) 193 (100) 105 (100) 33 (17.09) 15 (14.28)	MILD 193 (59.93)MODERATE 105 (32.60)SEVERE 24 (7.45)35 (18.13)25 (23.80)7 (29.16)21 (10.88)19 (18.09)5 (20.83)70 (36.26)90 (85.71)20 (83.33)7 (3.62)5 (4.76)1 (4.16)000165 (85.49)100 (95.23)22 (91.66)193 (100)193 (100)105 (100)24 (100)89 (46.11)65 (61.90)11 (45.83)193 (100)105 (100)24 (100)193 (100)105 (100)24 (100)33 (17.09)15 (14.28)9 (37.5)

Notes: Data are expressed as number (percentage), each percentage represents how often a specific variable is listed/described in reports; LA, left atrium; PAS pulmonary artery systolic; LVEDD, left ventricular end diastolic diameter; LV, left ventricle.



Table 4. Methods of MR assessment used.

METHOD OF ASSESSMENT USED n (%)	MILD 193 (59.93)	MODERATE 105 (32.60)	SEVERE 24 (7.45)	TOTAL	
"Eyeball" assessment	185 (95.85)	90 (85.71)	15 (62.5)	290 (90.06)	
Color flow imaging	47 (24.35)	35 (33.33)	8 (33.33)	90 (27.95)	
Vena contracta	0 (0)	5 (4.76)	1 (4.16)	6 (1.86)	
Flow convergence/PISA	0 (0)	4 (3.80)	3 (12.50)	7(2.17)	
Continuity equation	0 (0)	0 (0)	0 (0)	0 (0)	
Spectral continuous doppler	0 (0)	0 (0)	0 (0)	0 (0)	
Pulmonary venous flow	0 (0)	0 (0)	0 (0)	0 (0)	
Notes: Data are expressed as numbers (nercentage): PISA (proving) isovelecity surface area)					

Notes: Data are expressed as numbers (percentage); PISA (proximal isovelocity surface area).

by Carpentier et al.,¹² is necessary for appropriate management: type 1, normal leaflet (chordal) motion; type 2, leaflet prolapse, excessive chordal motion; and type 3, restricted leaflet or chordal motion.

The pathogenesis of MR is variable: degenerative disease (20–70%), rheumatic heart disease (3–40%), ischemic heart disease (13–40%), and infectious endocarditis (10–12%).⁷ In the studied population, there were 160 (49.68%) cases of MR without specified pattern; we estimate that MR pattern was not sufficiently provided, also etiologic and mechanistic description were insufficient (Tables 2 and 3). Of note, the same etiology of MR may yield regurgitation via different mechanisms (eg, congestive heart failure may yield MR via annular dilatation and/or via leaflet tethering). In the present study, we consider that a descriptive assessment of mitral leaflets morphology and dynamics should be performed more frequently for a better categorization of MR pattern. Consequently, the reported higher rate of functional MR compared with organic MR is not conclusive given the high rate of nonspecified patterns.

The prevalence of MVP in the general population is reported as 2.4%, and myxomatous degeneration of leaflets and chordae tendineae is the most common cause.^{13,14} In this study, MVP was reported in 4.5% of patients. MVP is better evaluated using the parasternal long-axis view; the apical four-chamber view may yield false-positive results given the saddle-shaped form of the annulus.^{5,15} Moreover, MV annulus measurements must be performed in telediastole,⁵ given that annular area decreases by nearly 25% during systole.^{14,16}

Jet characteristics (eg, central, eccentric, and multiple jets) were provided in up to 89.13% of cases, and we consider this a relatively satisfactory rate of jet reporting. Interpapillary distance was never cited in any report, though LV dilatation/ remodeling may suggest increased interpapillary distance; increased interpapillary distance (>20 mm) suggests a functional origin of MR.¹⁷

Echocardiographic assessment of MR. In order to assess the severity of MR, a talented sonographer uses several methods, when necessary, to comprise an integrated approach. In order to avoid subjective assessment and to decrease interobserver variability, the implementation of a specific protocol that conforms to recent guidelines is necessary.^{5,18,19} In this respect, the role of the echocardiography director to implement standardized protocols is of the utmost importance.²⁰

"Eyeball" assessment and color Doppler jet. "Eyeball" assessment of MR is subjective, and while it is valid for MR diagnosis and also for mild MR assessment when the jet is minimal and central,^{4,21} assessment of more severe MR requires more objective methods. Color Doppler using jet area to left atrial area ratio is a semi-quantitative method; a ratio of <20%, 20-40%, and >40% represents mild, moderate, and severe MR, respectively.8 Of note, the mechanism of MR influences the extent and form of the jet, that is, jets are often eccentric in organic MR, and jet area tends to underestimate MR severity in these cases; this is known as the Coenda effect.²² Conversely, in functional MR, jets are usually central with a slit-like orifice, and MR severity tends to be overestimated in these cases.²³ Accordingly, recent guidelines state that grading MR by "eyeballing" or by color Doppler jet area is not sufficient: "The color flow area of the regurgitant jet is not recommended to quantify the severity of MR. The color flow imaging should only be used for diagnosing MR."5 In the present study, "eyeball" and "color flow jet" methods were used in 90.06% and 27.95%, respectively, for both MR diagnosis and grading.

Vena contracta. Classically, a vena contracta width <3 mm indicates mild MR, whereas a vena contracta width $\geq 7 \text{ mm}$ reflects a severe MR.^{24,25} Importantly, intermediate values are not valid to distinguish moderate from mild or severe MR because of significant overlap. Accordingly, such values require the use of a complementary method for confirmation.⁵ The vena contracta method is valid for central and eccentric jets; however, this method is not accurate for assessment of MR caused by MVP with telesystolic regurgitant jet.²⁶ Also, it is not accurate for MR assessment when there are multiple jets. In the present study, vena contracta was rarely used (1.86%). In the present study, five patients were evaluated as having "moderate" MR without specifying whether complementary methods were used for confirmation.

Flow convergence method. The flow convergence method uses the proximal isovelocity surface area and it is by far the most recommended quantitative approach for



moderate and severe MR.⁵ It allows measurement of the effective regurgitant orifice area (EROA), regurgitant fraction, and regurgitant volume (RVol). The flow convergence method has a limited value in MR consecutive to MVP.²⁶ Moreover, in functional MR, there may be a dynamic variation of the regurgitant orifice, leading to underestimation of the EROA. Similarly, this method is not valid for evaluating eccentric jets, multiple jets, or complex regurgitant orifices.²⁷ In the present study, use of the flow convergence method was just 2.17%, far below the recommended rate of implementation.

Other echocardiographic methods for MR assessment. These are complementary methods, less frequently used in clinical practice, yet providing additional value in the context of an integrative approach, especially when the previous methods yield equivocal results. The continuity equation (Doppler volumetric method) is a time-consuming approach and is not recommended as a first-line method to quantify MR severity.¹⁹ Of note, a peak E velocity >1.5 m/s and/or a time velocity integral ratio (mitral/aortic) >1.5 suggests severe MR, in the absence of mitral stenosis. Similarly, the venous pulmonary flow has an additional value in MR assessment: a flow reversal is a strong marker of severe MR. Finally, a dense signal of the regurgitant jet with a full envelope using spectral continuous Doppler indicates more severe MR than a faint signal.¹⁹ In the present study, these methods were not mentioned in any echocardiographic report.

Advanced echocardiographic techniques. Transesophageal and three-dimensional echocardiography are reasonable tools to provide additional information in patients with complex MV lesion.^{16,24} However, these techniques are not indicated for MR assessment when a good quality transthoracic echo is available.¹⁹ Moreover, exercise echocardiography is a useful tool for MR assessment in special settings, especially in ischemic MR,²⁸ given that both orifice size and pressure gradient are dynamic and depend on loading conditions. Importantly, exercise echocardiography may provide a prognostic value; an increase in EROA \geq 13 mm² during exercise is a predictor of cardiac death.⁸

Clinical Implications

Herein, we aim to extend clinical awareness and practice to what is technically feasible for a better clinical outcome. To that end, we consider that self-assessment of real practice compared with the latest guidelines is a transparent and useful procedure to make progress. In addition, reporting such institutional experience may be useful to some "similar" institutions considering their echocardiography practice.

In this context, we re-emphasize the value of accurate assessment of MR, implementing a quantitative and/or integrative approach for every "more than mild" grade, and awareness of the strengths and weaknesses of every method. Accurate assessment is of utmost importance, given its potential impact on prognosis and management options from careful watching to MV repair or replacement. For instance, severe MR as assessed with the flow convergence method (organic MR, EROA \geq 40 mm² and RVol \geq 60 mL; functional MR, EROA \geq 20 mm² and RVol \geq 30 mL) is an indicator of poor prognosis.⁸ Moreover, ischemic MR, even when it is mild, is associated with higher mortality, with a graded relationship between severity and reduced survival.²⁹

As the long-term prognosis of suboptimal MV repair is worse than that of valve replacement, it is crucial to define clearly the underlying pathology of MR together with quantification of its severity. The most frequently used approach for repairing functional MR is restrictive annuloplasty; however, its major drawback is the recurrence of MR when performed alone in patients with extensive leaflets tethering or with complex MR jets.³⁰ New alternative procedures targeting the subvalvular apparatus and the LV may be considered for patients with severe leaflets tethering under the guidance of echocardiographic features of MV apparatus, otherwise such patients are more suitable for MV replacement.^{31,32} Successful repair is also feasible for MR due to prolapsing leaflets (particularly the posterior leaflet) when echocardiographic features are favorable for repair (ie, absence of severe leaflet remodeling) and this is recommended even before the apparition of symptoms and/or left ventricular enlargement.6

In the present study, more than one-third of patients (36.43%) had their echo requested by non-cardiologists. The question to raise here is whether they are referred later to a cardiologist if they were diagnosed with significant MR. The echocardiographic report should give potential clues as to the likelihood of MV repair.^{15,18} In fact, MV repair should be favored over MV replacement. However, such decisions must take into account technical feasibility and the level of experience at the surgical center.³³ The purpose is beyond diagnosing and reporting MR; it is also beyond the whole echocardiographic product. The ultimate objective is to improve clinical outcome by means of appropriate management.

Perspectives and Quality Improvement Program

While mapping the present, thinking globally and applying locally, in order to remodel the future, a quality improvement project must aim to change our practice; to do this, it must be applicable and regularly updated. Despite the potential behavioral complexities and reporting diversities among sonographers, implementation of a uniform assessment protocol helps to minimize intra- and interobserver variabilities and discrepancies. In addition, assessment of clinical outcome and positive interaction with the referring physician are important steps beyond the echocardiographic product.

Moreover, making full use of available technology is essential before implementing new technologies (threedimensional echo, deformation imaging, stress echo, etc.). Otherwise, new technology may lead to a disruptive progress rather than to an evolutionary process. A quality improvement project must aim to create a procedure requiring specification of the indications of echocardiography for all patients, more frequent recording of MR pattern, and also an algorithm conforming to the latest guidelines to be implemented for diagnosis of MR severity. Such a project must be re-modeled regularly and must be applicable locally. Management decisions must be based on teamwork, mostly represented by medico-surgical scientific meetings, in order to make appropriate decisions based on the latest recommendations and taking into account local capabilities. Such a quality improvement project was created and implementation was initiated in collaboration with the medical direction.

Study Limitations

This study is retrospective and monocentric, and this may compromise results. Echocardiography images are best viewed as moving pictures, with preferably the motion of a full cardiac cycle; in this study, most images were reviewed as static images, which also may compromise assessment.

Physiological MR was not reported in this study, and we hypothesize that most cases of less than mild MR were labeled as mild MR. According to recent guidelines,^{5,18,19} accurate MR grading requires the use of quantitative methods, preferably with an integrative approach. Consequently, we estimate that the labeled MR severity in this study – when assessed with qualitative or semi-quantitative methods – may not represent the real grade of MR.

Conclusion

MR prevalence was reported at 33.50%, and a functional MR pattern was mostly reported. However, MR pattern was not specified in almost half of the cases. "Eyeball" and "color flow jet" methods of assessment were mostly used; these methods are adequate for MR diagnosis and for assessment of "physiological" or "mild" MR with small central regurgitant jet. However, any "more than mild" MR requires implementation of quantitative methods, as stated by recent guidelines. After this self-assessment process, a quality improvement project was formed, and implementation has been initiated with the cooperation and involvement of the medical director in the facility.

Acknowledgement

The authors would like to express their gratitude to Professor L Elif Sade (Chair Working Group on Echocardiography, Turkish Society of Cardiology, Ankara, Turkey) for the constructive remarks and comments brought to this paper.

Survey Objectives and Clinical Practice

Such survey, assessing local practice and comparing it to the latest guidelines, aimed to extend awareness and to improve clinical outcome, like any institutional or national survey published in the medical databases. The methodology described in this paper- which is not fully concordant with latest guidelines- may be potentially encountered in some local, regional



or distant facilities; accordingly, the authors do not imply herein any judgment of any individual or collective practice in any healthcare system. This survey has purely and simply an educational and research objectives, while extending clinical awareness in order to improve clinical practice. Interestingly, such study corroborates the full implementation of the available techniques and equipment before applying for the latest technology and this issue is of utmost importance for the Administrative Board in any medical facility. Moreover, and at all times, it is the professional responsibility of the practitioner to apply independent judgment on which methodology is better applicable in every particular case.

Author Contributions

Conceived and designed the experiments: AK. Analyzed the data: AK, VA. Wrote the first draft of the manuscript: AK, VA. Contributed to the writing of the manuscript: AK, VA. Agree with manuscript results and conclusions: AK, VA. Jointly developed the structure and arguments for the paper: AK, VA. Made critical revisions and approved final version: AK, VA. Both authors reviewed and approved of the final manuscript.

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